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Collection of airborne spores by circular single-stage impactors with small jet-to-plate distance

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Abstract

Most of the commonly used bioaerosol samplers are single-stage impactors that meet the conventional Marple's design criteria: their non-dimensional jet-to-plate distance, S/W, is greater than the established threshold (1.5 for rectangular nozzles and 1 for the circular ones). Recent studies have shown that these samplers underestimate the concentration of airborne fungal spores because their cut-off size is about 2.5 μm (Air-O-Cell and Burkard samplers) or greater while some fungal species produce spores of ca. 1.8–2.5 μm in aerodynamic diameter. In this study, we evaluated the single-stage circular-jet impactors with very small jet-to-plate distances ($S/W \ll 1$). The laboratory and field data obtained with test particles of different sizes and different origin (biological and non-biological) demonstrated the feasibility of these "incorrectly designed" impactors for the spore collection and total enumeration (viable + non-viable spores). A decrease in the jet-to-plate distance resulted in a critical decrease of the impactor's cut-off size (d_{50}): from 2.5 μm to about 1 μm. This reduction of cut-off size makes such an impactor efficient for collecting spores of all fungal species ($\geqslant 1.8$ μm) and even some bacterial species ($\geqslant 1$ μm). Since the spore surface density across the circular deposit area was non-uniform, three sample reading procedures were evaluated: the entire area count, random partial count, and a partial count on a rectangular "diametric slice". The collection efficiency data suggested that a relatively small jet-to-plate distance is likely to result in excessive shear forces in the impaction zone, thus enhancing the spore deaggregation and bounce. The coefficient of inter-sample

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variation of the field samples, collected by commercially available impactors with $S/W \approx 0.099$, did not exceed 50% for the total spore count. The highest variability was observed for Arthrospores, which were more aggregated than other types of fungi.

Keywords: Impactor; Bioaerosol; Collection efficiency; Jet-to-plate distance

1. Introduction

Exposure to high levels of indoor and outdoor bioaerosols, particularly to airborne spores, is associated with a risk of respiratory infections, various allergic responses, and other adverse health effects (Cox & Wathes, 1995). Assessment of the potential hazards caused by the bioaerosol exposure requires accurate collection of representative samples and their quantitative and qualitative characterization. A large variety of methods for bioaerosol sampling are available, including impaction, impingement, collection on filter, and electrostatic precipitation. Inertial impactors have some limitations for measuring viable bacteria and fungi because high impact velocity can result in the metabolic and structural injuries of the collected microorganisms (Stewart, Grinshpun, Willeke, Terzieva, & Ulevicius, 1995; Terzieva et al., 1996; Lin, Reponen, Willeke, Grinshpun, & Foarde, 1999). These limitations do not apply to the enumeration of total airborne microorganisms (viable + non-viable count). The airborne spore enumeration is usually conducted after collecting the spores on a slide of a single-stage impactor. The Burkard 7-day Volumetric Spore Trap (Burkard Manufacturing Company Ltd., Hertfordshire, UK) with a single-stage rotating drum is widely used for collecting outdoor fungi (Emberlin, Newman, & Bryant, 1995; Mitakakis, Clift, & McGee, 2001). The slit glass-slide impactors, such as the Burkard Personal Volumetric Air Sampler (Burkard Manufacturing Company Ltd.) and the Air-O-Cell sampling cassette (Zefon Analytical Instruments, Inc., St. Petersburg, FL, USA), are typically used for indoor spore sampling (e.g., Levetin, Shaughnessy, Fisher, Ligman, & Harrison, 1995; DeKoster & Thorne, 1995; Adhikari, Sen, Gupta-Bhattacharya, & Chanda, 2000).

While all single-stage inertial bioaerosol impactors operate on the same principle, their collection efficiency may be drastically different. The efficiency of an impactor is characterized by the cut-off size, d_{50}, defined as the particle aerodynamic diameter, d_a, for which the impactor's collection efficiency is equal to 50%. For example, the cut-off size of the Burkard Personal Volumetric Air Sampler and the Air-O-Cell sampler at their standard operating flow rates ($Q = 10$ and 15 L min^{-1}, respectively) is approximately 2.5 μm (Reponen, Willeke, Grinshpun, & Nevalainen, 2001b; Trunov, Trakumas, Willeke, Grinshpun, & Reponen, 2001). This cut-off size makes the above impactors efficient for collecting spores of *Penicillium melinii* with $d_a \approx 3$ μm (Reponen, Grinshpun, Conwell, Wiest, & Anderson, 2001a) and *Stachybotrys* spp. with $d_a \approx 4.2$ μm (Reponen, 1995). Our earlier studies (Aizenberg, Reponen, Grinshpun, & Willeke, 2000; Grinshpun, Reponen, Willeke, Mainelis, Górny, & Trunov, 2002) showed that these samplers are less efficient (< 50%) in collecting spores of smaller sizes, such as *Bacillus subtilis* var. *niger* with $d_a \approx 0.9$ μm (Aizenberg et al., 2000) or *Cladosporium cladosporioides* with $d_a \approx 1.8$ μm (Reponen et al., 2001a).

Fungal species produce primarily spores with an aerodynamic diameter of 1.8 μm or greater. Therefore, the current instrument development effort is directed toward reducing the cut-off size of an impactor below 1.8 μm, which would make such an impactor efficient for collecting most fungal species. Furthermore,

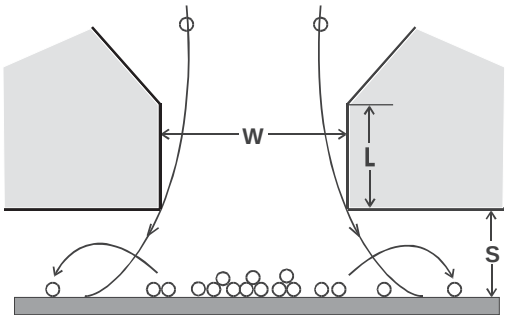

Fig. 1. Schematic representation of a single stage impactor.

some designs aim at achieving $d_{50} < 1\,\mu m$ so that the impactor would be efficient not only for fungi but also for many spore-forming bacterial species. Examples are the three novel single-stage impactors, namely CyClex BioAerosol Impact Sampler, Cyclex-d Cassette, and Micro5 Microcell, which have recently become commercially available (all samplers are manufactured by EMS, Inc., Charleston, SC, USA). These impactors have a circular nozzle (unlike the rectangular one of the Burkard and Air-O-Cell samplers). The major novelty, however, is in the jet-to-plate distance, *S*, which for all three EMS impactors is considerably lower than their nozzle diameter, *W* (see Fig. 1).

Resulting from extensive theoretical analyses and experimental studies, the conventional single-stage impactors are usually designed based on two criteria (Marple, 1970; Marple & Liu, 1974; Marple & Willeke, 1976a,b; Rader & Marple, 1985):

(1) the non-dimensional jet-to-plate distance, *S/W*, should be larger than 1 for circular inlets and larger than 1.5 for rectangular ones; and
(2) the Reynolds number, *Re*, of the air flow in the nozzle (throat) should be between 500 and 3000 for both circular and rectangular inlets.

The above criteria produce the desirable sharp collection efficiency curve with a cut-off size that is not sensitive to a small variability of the jet-to-plate distance. In addition, the spatial uniformity of the particle deposit on the collection plate is improved if the limiting conditions for *S/W* and *Re* are met. The latter may be preferable in a situation when the microscopic count is not performed on the whole deposit area but—instead—on a part of this area (the partial slide reading).

Marple (1970) studied the collection efficiency as a function of the impactor's characteristic Stokes number, Stk. The collection efficiency curves of the impactors are often characterized by Stk_{50}, or the Stokes number that provides the collection efficiency of 50%:

$$Stk_{50} = \frac{\rho_p C_c d_{50}^2 U}{9\eta W}, \tag{1}$$

where ρ_p is the particle density; C_c is the Cunningham correction factor ($\sim$1 for particles larger than 1 μm); U is the airstream velocity in the nozzle; and η is the air viscosity. The Marple's study demonstrated that the circular-jet design of the impactor allows achieving greater collection efficiency than the rectangular-jet design (at the same Stk-values). Furthermore, the efficiency curve of the circular-jet impactor appeared

to be less sensitive to the jet-to-plate distance limitation than that of the rectangular one, given that both were operating under the same conditions. For instance, if the S/W criterion for circular impactor is not met, i.e., $S/W \leqslant 1$, the collection efficiency curve becomes less sharp as the non-dimensional jet-to-plate distance decreases. On the other hand, this curve shifts to the left allowing better collection of smaller particles (Marple, 1970). For example: $\mathrm{Stk}_{50} \approx 0.226$ at $S/W = 1$, while $\mathrm{Stk}_{50} \approx 0.075$ at $S/W = 1/8$ (the lowest tested value in the Marple's study). The above change in the S/W-ratio decreases the cut-off size by a factor of $(0.226/0.075)^{0.5} = 1.74$.

Generally, the sharp "cut-off" is important from the particle size classification standpoint. The step-function collection efficiency curve assures that all particles with $d_a > d_{50}$ are collected and all those with $d_a < d_{50}$ pass through (Hinds, 1999). However, if an impactor is designed to collect particles of a specific nature (e.g., spores, which are usually distinct from other particles when being observed and counted under the microscope), the primary requirement is only to assure that all the particles within the size range of interest are collected at the efficiency greater than 50%. Therefore, this study was initiated to experimentally determine the collection efficiency of circular single-stage impactors that have very small jet-to-plate distances, $S/W \ll 1$, i.e., do not meet the first impactor design criterion. The applicability of these "incorrectly designed" impactors was investigated with respect to the airborne spore collection on the slide followed by the slide microscopic analysis.

2. Experimental

2.1. Laboratory evaluation

2.1.1. Experimental setup

The experimental setup is shown in Fig. 2. This facility has been used in our previous studies of bioaerosol samplers (Stewart et al., 1995; Terzieva et al., 1996; Aizenberg et al., 2000; Trunov et al., 2001). The test particles were aerosolized as described below and then diluted with HEPA-filtered compressed air. The diluted aerosol passed through a 10-mCi ^{85}Kr electrostatic charge equilibrator (model 3012, TSI Inc., St. Paul, MN, USA) and then entered the aerosol chamber (approximately 14.5 cm in diameter and 25 cm in height), which housed the tested impactor. The aerosol concentration, C_{UP}, upstream of the impactor, and the concentration, C_{DOWN}, downstream of it, were measured using an optical particle size spectrometer (Model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA) operated at a flow rate of 1.2 L min^{-1}. The Grimm OPC, equipped with an inlet that had the same design as the impactors' inlet, measured particles in the optical size range of 0.3 to > 10 μm. The data were recorded in 15 channels with a time resolution of 6 s. The particle size distributions and concentrations were also measured by an aerodynamic particle size spectrometer (Aerosizer, TSI Inc. - Amherst Process Instruments, Hadley, MA, USA) operated at a flow rate of 5.1 L min^{-1}. When liquid particles, such as oleic acid, are accelerated in the Aerosizer's nozzle, they become elongated. Hence, the instrument does not generally measure the true diameter of spherical particles. Based on the findings published by Baron, Martinez, and Jones (2003) and Bartley, Martinez, Baron, Secker, and Hirst (2000), we estimated the problem of incorrect particle size measurement due to droplet distortion and found that it occurs only when the oil droplets are larger than 4–5 μm. Since our test particles were smaller, the Aerosizer data were not corrected for the above effect. The relationship between the optical and aerodynamic particle diameters obtained with the Grimm OPC and the Aerosizer, respectively, was established.

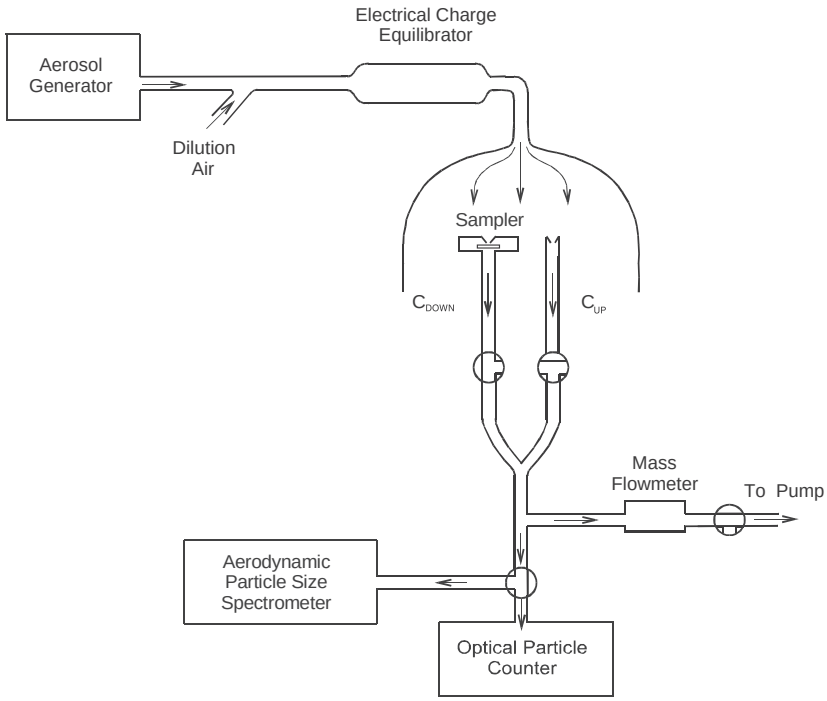

Fig. 2. Experimental setup.

The entire setup was located in a class II biosafety cabinet, (Model 6TX, Baker Co., Inc., Sanford, ME, USA). The temperature was maintained at 23±1 °C and the relative humidity at 35 ± 5% throughout the experiments.

2.1.2. Test aerosols

The performance characteristics of the impactors were studied with physical and biological particles of different sizes. The physical aerosols included polydisperse oleic acid liquid droplets ($d_a = 0.5$–$5\,\mu m$) and monodisperse polystyrene latex (PSL) spheres ($d_a = 0.85$, 1.18, 1.53, 2.20, and 3.04 μm, obtained from Bangs Laboratories, Inc., Fishers, IN, USA). The biological aerosols chosen for the collection efficiency experiments were: *Bacillus subtilis* var. *niger* [BG, bacterial spores of $d_a = 0.9\,\mu m$ ($\sigma_g = 1.2$) obtained from the US Army Edgewood Laboratories at the Edgewood Research, Development, and Engineering Center, Aberdeen Proving Grounds, MD, USA; courtesy of Ms. A. Akiyemi and Dr. E.W. Stuebing], *Cladosporium cladosporioides* [fungal spores of $d_a = 1.8\,\mu m$ ($\sigma_g = 1.1$), ATCC 58991], *Aspergillus versicolor* [fungal spores of $d_a = 2.5\,\mu m$ ($\sigma_g = 1.1$), RTI Culture # 367; obtained from the Research Triangle Institute, NC, USA; courtesy of Dr. E. Cole]. These bacterial and fungal species are common in indoor and outdoor air environments (Meldrum, O'Rourke, Boyer-Pfersdorf, & Stetzenbach, 1993; Madelin, 1994; Horner, Helbling, Salvaggio, & Lehrer, 1995; ACGIH, 1999). The BG spores are also commonly used as a simulant of *Bacillus anthracis* and several other biological agents (Johnson, Martin, & Resnick, 1994; Franz, Parrott, & Takafuji, 1997; Hill et al., 1999). In addition, for some selected experiments, *Penicillium brevicompactum* [fungal spores of $d_a = 2.3\,\mu m$ ($\sigma_g = 1.1$), CBS 480-84] and

Penicillium melinii [fungal spores of $d_a = 3.0\,\mu m$ ($\sigma_g = 1.1$), isolated from a moldy building] were used as test microorganisms. All fungal cultures utilized in this study were between 1 and 2 weeks old, which is generally sufficient for mature sporulation (Burge, 1995).

Before their generation, *B. subtilis* endospores were washed twice with sterile de-ionized water by centrifugation at 7000 rpm for 7 min. *C. cladosporioides* and *A. versicolor* were cultured on malt extract agar (Beckton Dickinson Microbiology Systems, Sparks, MD, USA). Dichloran Glycerol (DG 18) agar (Beckton Dickinson Microbiology Systems) was used to culture *P. brevicompactum* and *P. melinii*. The fungal cultures were incubated before experiments at 25 °C for 7–14 days.

Depending on the type of the test particles, different aerosol generators were utilized. A Collison nebulizer (BGI Inc., Waltham, MA, USA), operated at $4\,L\,min^{-1}$, was used to generate polydisperse oleic acid and monodisperse PSL particles, as well as *B. subtilis* spores from a de-ionized water suspension containing no surfactant. The strong shear forces created by the nebulizer's high-velocity air jet enhanced deagglomeration, thus reducing the percentage of large spore aggregates. Fungal spores were aerosolized directly from their growth agar using an agar-tube disperser (Reponen, Willeke, Ulevicius, Grinshpun, & Donelly, 1997) operating at $15\,L\,min^{-1}$. This spore generator consists of a 30-cm long cylindrical tube with an orifice at each of the opposite ends. The fungi grown on the agar surface in a half-cylindrical container are placed inside the dispersion tube. The agar-tube disperser simulates the natural release of dry spores into the air and reduces the percentage of agglomerated airborne spores due to considerable shear forces inside the exit orifice.

2.1.3. Test impactors

In this study, we tested single-stage circular-jet impactors with a conical-shape nozzle (angle $= 34°$). The first test impactor (I-1) was made of stainless steel with an impaction orifice of $W = 4.62$ mm diameter and a nozzle (throat) length of $L = 11.9$ mm. A slide covered with a particle-bounce-preventing adhesive served as an impaction plate (provided by the EMS, Inc.). The sampling flow rate was $Q = 20\,L\,min^{-1}$. Impactor I-1 was designed with an adjustable jet-to-plate distance and evaluated in four configurations representing the following non-dimensional jet-to-plate distances: $S/W = 0.033$, 0.066, 0.099, and 0.13. One of the above configurations ($S/W = 0.099$) is utilized in the newly developed commercial spore impactors, namely the CyClex BioAerosol Impact Sampler (steel body) and Cyclex-d Cassette (plastic body). Test impactor I-2 was made of plastic with an impaction orifice diameter, $W = 2.15$ mm, approximately twice smaller than that of I-1. The sampling flow rate of I-2, $Q = 5\,L\,min^{-1}$, was four times lower than the flow rate of I-1. Thus, these impactors had about the same air velocity through the nozzle (throat). Designed with a fixed jet-to-plate distance ($S/W \approx 0.099$), impactor I-2 is similar to the commercially available Micro5 Microcell spore impactor. Both impactors, I-1 and I-2, utilized similar adhesive-covered plastic slides. The jet-to-plate distances were determined with a tolerance of $\approx 25\,\mu m$.

2.1.4. Determination of the collection efficiency

The performance of an aerosol sampler collecting airborne microorganisms depends on its inlet characteristics and collection efficiency (Nevalainen, Pastuszka, Liebhaber, & Willeke, 1992; Grinshpun, Chang, Nevalainen, & Willeke, 1994). The errors associated with the particle aspiration into the impactor's inlet and the internal wall losses in impactors I-1 and I-2 are negligibly small for $d_a \leqslant 10\,\mu m$ (Grinshpun, Willeke, & Kalatoor, 1993). The inlet sampling efficiency of the test impactors was 100±5%. Using the particle fractional concentrations measured by the Grimm OPC upstream and downstream of the sampler,

the overall collection efficiency (or capture efficiency), E_C, was determined for each optical particle size as follows:

$$E_C = \left(1 - \frac{C_{DOWN}}{C_{UP}}\right) \times 100\%. \quad (2)$$

The optical particle size scale was converted to the aerodynamic one through the comparison of the particle size distributions measured by the Grimm OPC and the Aerosizer. The particle density used for the Aerosizer's data manipulation program was set at 0.8 g cm^{-3} for oleic acid particles, 1.05 g cm^{-3} for PSL spheres, 1.0 g cm^{-3} for bacterial spores (Bakken & Olsen, 1983), and at 1.1 g cm^{-3} for fungal spores (Gregory, 1973).

The sampling time, t, during which the particle concentrations and size distributions upstream and downstream of each impactor were measured, ranged from 1 to 10 min. When testing with the oleic acid or PSL particles, each experiment was repeated three times. When testing with spores, each experiment was repeated eight times (because of the greater variability in the spore size and concentration distributions).

After the capture efficiency was determined for spores by optical particle counting, the slides were analyzed using a microscope with phase-contrast objectives (Model Labophot 2, Nikon Corp., Tokyo, Japan) at a magnification of 400× or 1000×. First, the total number of spores, $N_{MICROSCOPE}$, collected on the slide deposition area, A_{DEP}, was determined. Since some spores were agglomerated, it was important to define the counting procedure. Some guidelines recommend counting an aggregate that consist of n spores as one unit (count of spore propagules), while others recommend counting it as n units (single spore count). The microscopic enumeration was performed using both methods. The relationship between the propagule and single counts was used to characterize the percentage of spore aggregates.

To determine the actual collection efficiency of the tested impactor, E_{ACTUAL}, the number of propagules, $N_{MICROSCOPE}$, counted under the microscope was related to the upstream bioaerosol concentration of the impactor, C_{UP}:

$$E_{ACTUAL} = \frac{N_{MICROSCOPE}}{C_{UP} Q t} \times 100\%. \quad (3)$$

The direct-reading aerosol measurement (both by the Grimm OPC and the Aerosizer), as well as the propagule count under the microscope, identifies a spore agglomerate as one particle. Therefore, when calculating E_{ACTUAL}, we utilized $N_{MICROSCOPE}$ data that were based on the propagule count. Generally, we perceive E_{ACTUAL} as a performance characteristic of a bioaerosol sampler complementary to the E_C. The latter is affected by the particle losses inside the sampler, so that as long as $E_{ACTUAL} < E_C$, E_{ACTUAL} seems to be more adequate performance characteristic of a bioaerosol sampler. However, the definition represented by Eq. (3) it is not perfect as the particle deagglomeration on the impaction plate may artificially increase the numerator, so that the actual collection efficiency may even exceed 100%.

2.1.5. Spore uniformity on the slide

The spore enumeration on the slide was conducted after mounting it with the lactophenol cotton blue stain (Beckton Dickinson Microbiology Systems) using a 25-mm^2 cover glass. The edges of the cover glass were sealed by transparent nail enamel. This staining technique allowed differentiating unpigmented hyaline spores. In addition, phase contrast microscope objectives were used to improve the contrast

between the particles and the background. The spore enumeration was performed using the Model Labophot 2 Nikon microscope and a high-resolution digital image analysis system (SPOT RT Diagnostic Instruments, Fryer Company, Cincinnati, OH, USA).

There are several different ways of determining $N_{\mathrm{MICROSCOPE}}$ from the spore microscopic count on the slide. The entire slide is rarely counted because such a procedure is too time-consuming. In practice, the spores are often enumerated on a certain number of microscopic fields (at least 40 fields or 400 particles are usually recommended). Then the average number of spores per field is multiplied by the ratio of the entire deposit area to the microscopic field area, $A_{\mathrm{DEP}}/A_{\mathrm{FIELD}}$. This assumes that the particle surface density is relatively uniform across A_{DEP}. For many slit impactors, including the Burkard Personal Volumetric Air Sampler and the Air-O-Cell cassette, the particles are not uniformly collected on the slide exhibiting considerable variability of the microscopic counts across the two-dimensional deposit area (Aizenberg et al., 2000). For a single-stage, circular-jet impactor (especially the one with a small jet-to-plate distance), the particle surface density may significantly vary across the circular deposit area, from the center to the periphery. Thus, three counting methods were used to develop the partial count procedure on the non-uniform deposit area that would sufficiently represent the sample. First, the total count was performed on the entire circular deposit area, $A_{\mathrm{DEP}} = \pi D_{\mathrm{DEP}}^2/4$. The definition of A_{DEP} was not trivial since the deposit on the slide had no clear borderline. A_{DEP} was determined based on the microscopic slide observation by scanning through the center of the deposit from $-W$ to $+W$. The diameter of the deposit area, D_{DEP}, was usually between W and $1.5 \times W$. Second, the total spore count was conducted on 10–20 microscopic fields randomly chosen inside D_{DEP} (the computer-generated random numbers were transferred to the coordinate system within the deposit area). Third, the spores were counted on a rectangular "diametric slice" (strip) across the deposit area. The "slice" length was about D_{DEP} (closest multiple of microscopic fields) and its width was equal to the width of a single microscopic field, as presented in Fig. 3. The average number of spores per microscopic field was obtained with each of the three counting procedures applied to the slide. Thus, the representativeness of the "slice" counting procedure was evaluated as compared to the random microscopic count and the entire area count.

2.2. Field evaluation

Following the laboratory evaluation, three commercially available impactors with $S/W < 10^{-1}$, were tested in the field in two separate experiments.

First, the CyClex BioAerosol Impact Sampler ($Q = 20\,\mathrm{L\,min^{-1}}$, $S/W = 0.099$) was used to collect samples in six sites in Southwest Ohio (five indoor environments and one outdoor environment). All indoor sites were characterized by the presence of visible mold. The sampling time was 3–15 min.

Following the sampling, each slide was mounted with the stain and prepared for microscopic analysis following the protocols described in Section 2.1.4. The total spore enumeration was performed under the 400× magnification of a Leitz Laborlux S microscope (W. Nuhsbaum, Inc., McHenry, IL, USA). Two procedures were used: (i) counting on 20 microscopic fields randomly chosen inside the deposit area (the total number of spores exceeded 400) and (ii) counting on the rectangular "diametric slice". The average number of spores per microscopic field was determined for the "random" and "slice" counts, respectively. The relative difference between them was calculated for each sample to evaluate the representativeness of the "slice" counting procedure, as compared to the random microscopic count. The entire area count

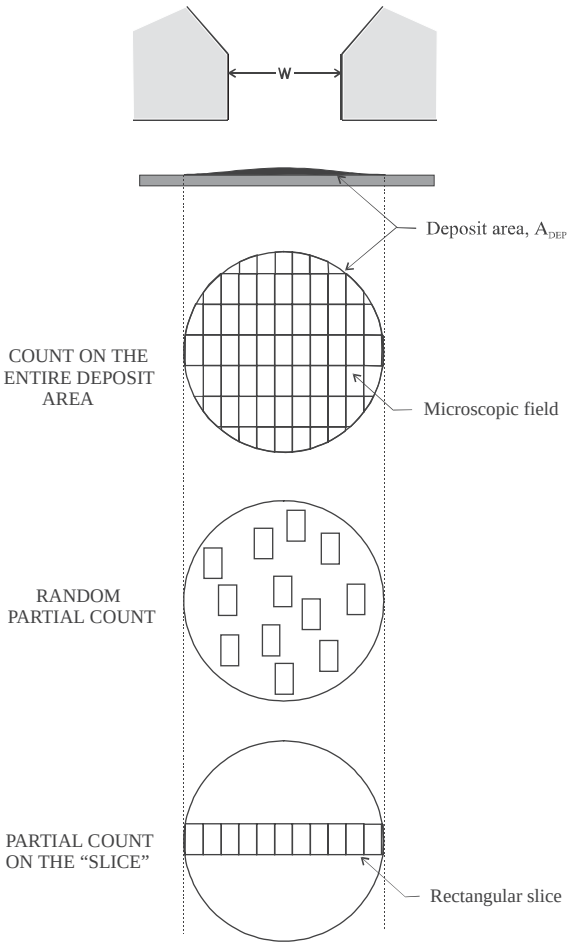

Fig. 3. Schematic representation of the microscopic counting procedures.

was not performed at this phase since the previous tests showed that the "random" count was sufficiently representative.

Second, three identical circular slit impactors of each type, including 3× CyClex and 3× Cyclex-d (with the same Q and S/W), and 3×Micro5 (Q=5 L min^{-1}, $S/W \approx 0.099$), were used to collect samples in two indoor sites previously identified as mold-problem environments (one in Ohio and one in South Carolina). In each test, three identical samplers of a specific type operated in parallel for 3–15 min with three replicates. The total spore count and the genus/group-specific counts within 22 fungal spore types were performed for each aerosol sample. To characterize the precision of a specific sampler type with respect to the total and genus/group-specific counts, the coefficient of variation, CV, was determined for each set of the three simultaneously operated identical samplers ($m = 3$). For comparison, the spore sampling was performed in the same two field sites using three identical Air-O-Cell cassettes ($Q = 15$ L min^{-1}, $S/W = 1$), and the CVs of the total spore count was determined.

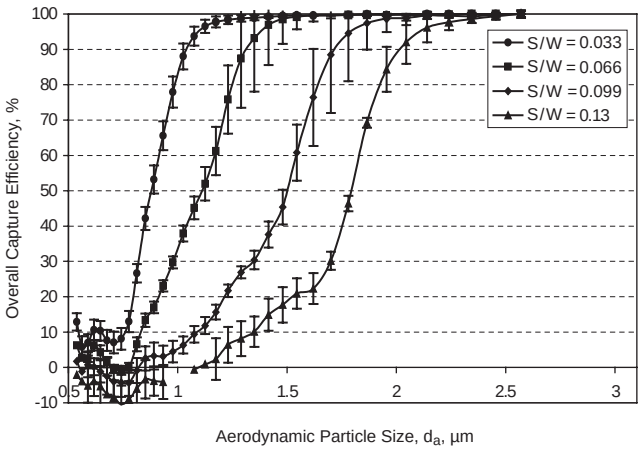

Fig. 4. Overall capture efficiency of impactor I-1 (four configurations) for oleic acid particles.

3. Results and discussion

3.1. Overall capture efficiency and actual collection efficiency

Fig. 4 presents the overall capture efficiency curves obtained with oleic acid particles collected by impactor I-1 for its four configurations. The difference between the aerodynamic and optical particle diameters of oleic acid particles (measured with the aerodynamic and optical particle size spectrometers, respectively) did not exceed 15%. As was anticipated, the overall capture efficiency was significantly affected by the non-dimensional jet-to-plate distance because the conventional jet-to-plate distance criterion of $S/W > 1$ for circular impactors (Marple, 1970) was not met. The efficiency sharply increased with the increasing particle diameter and asymptotically reached 100%. The data show that the lowest cut-off size, $d_{50} = 0.86\,\mu m$, was achieved for the smallest $S/W = 0.033$. At $S/W = 0.066$ and 0.099, the cut-off size increased to $d_{50} = 1.12$ and $1.5\,\mu m$, respectively. For the largest jet-to-plate distance ($S/W = 0.13$), $d_{50} = 1.8\,\mu m$. The first three configurations of impactor I-1 ($S/W = 0.033$, 0.066, and 0.099) assured cut-off sizes, which were low enough to anticipate that all airborne fungal species could be collected efficiently ($E_C > 50\%$). Furthermore, the smallest S/W may be suitable for collecting even smaller bioaerosol particles, such as bacterial spores, which are mostly about 1 μm in aerodynamic diameter. Among the tested configurations, the forth one (with the largest S/W) showed the lowest potential for the spore collection. Thus, further experimentation with impactor I-1 was done only with the three smallest jet-to-plate distances.

Following the tests with a polydisperse aerosol, E_C of impactor I-1 was determined for five monodisperse PSL particle fractions ranging from 0.85 to $3.04\,\mu m$, as presented in Fig. 5. The aerodynamic and optical particle diameters of PSL particles were essentially the same (within ±5%). The data generally confirmed the trends seen in Fig. 4. However, the E_C-values for PSL spheres were lower than those obtained with oleic acid particles. This difference can be attributed to the particle bounce effect, which is more pronounced for solid, elastic PSL spheres, as compared to liquid non-elastic oleic acid droplets, since the latter adhere well to a coated surface of the collection slide. Some PSL particles that impacted

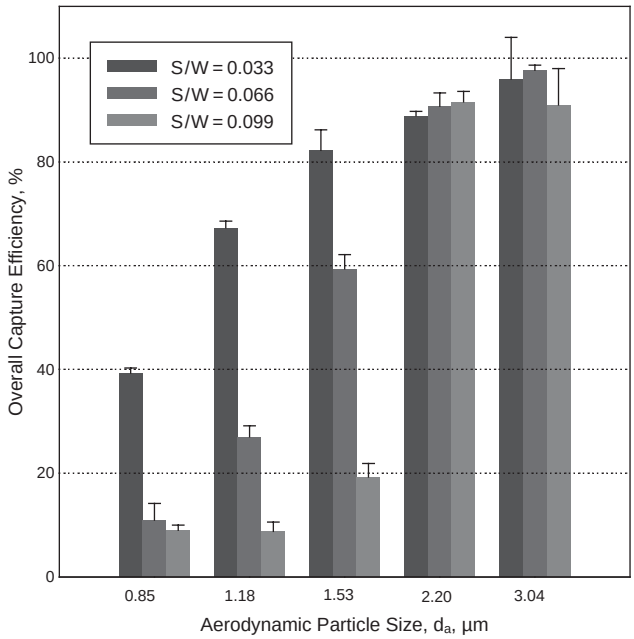

Fig. 5. Overall capture efficiency of impactor I-1 (three configurations) for PLS particles.

on the slide and bounced off could have been carried away by the effluent airflow and subsequently be detected downstream of the impactor, thus reducing the overall capture efficiency. Moreover, new incoming particles are likely to bounce from previously deposited ones. This effect may be more pronounced for higher particle concentrations.

It should be emphasized that the overall capture efficiency does not allow differentiating between the particles collected on the slide (inside of the collection area) and those, which were lost elsewhere in the impactor. From this viewpoint, E_C may overestimate the actual collection efficiency. On the other hand, E_C can be underestimated by Eq. (2) because the break-up of spore aggregates and their subsequent escape with an effluent air increases C_{DOWN} while C_{UP} remains the same. This occurs when an aggregate that is detected by the OPC as a single particle upstream of the impactor produces several particles as a result of the break-up, which—if not collected in the impactor—may be detected downstream, thus "artificially" decreasing the overall capture efficiency. Thus, the overall capture efficiency can generally be either greater or lower than the actual collection efficiency.

The tests performed with airborne spores allowed us to determine both the overall capture efficiency (through the aerosol particle count upstream and downstream of the impactor) and the actual collection efficiency (through the microscopic count on the slide). The results obtained for the three configurations of impactor I-1 ($S/W = 0.033$, 0.066, and 0.099) are presented in Table 1. As expected, submicron bacterial spores of *B. subtilis* were collected with rather low efficiency ($< 50\%$). At the same time, for larger fungal species ($\geqslant 1.8\,\mu m$), both E_C and E_{ACTUAL} were sufficiently high ($> 50\%$) to conclude that the tested impactors with a low jet-to-plate distance have a good potential for efficient fungal spore collection. The overall capture efficiency trends shown in Table 1 for bacterial and fungal spores are in a fair agreement with those, which were obtained using oleic acid and PSL particles of the same aerodynamic sizes (Figs. 4 and 5).

Table 1
The overall capture efficiency (E_C)[a] and the actual collection efficiency (E_{ACTUAL})[b] for the laboratory-generated microbial spores sampled by a single-stage impactor (I-1) with different jet-to-plate distances, *S/W*

Microorganism (d_a)	Efficiency	S/W		
		0.033	0.066	0.099
B. subtilis	E_C	22.7 ± 1.8	29.1 ± 3.6	22.2 ± 1.9
(0.9 μm)	E_{ACTUAL}	19.1 ± 4.1	19.1 ± 10.0	18.5 ± 3.7
C. cladosporioides	E_C	56.4 ± 10.0	60.0 ± 8.1	55.5 ± 10.2
(1.8 μm)	E_{ACTUAL}	69.1 ± 2.7	61.8 ± 3.6	67.5 ± 2.8
A. versicolor	E_C	89.1 ± 4.5	70.9 ± 23.6	87.0 ± 3.7
(2.5 μm)	E_{ACTUAL}	51.8 ± 12.7	54.5 ± 7.3	50.9 ± 13.0

[a]Determined by a real-time aerosol monitoring upstream and downstream the impactor.
[b]Determined using the microscopic enumeration of propagules (random partial count).

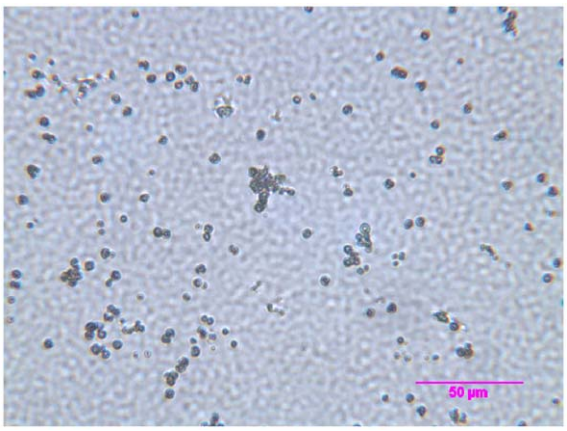

Fig. 6. Spores of the laboratory-aerosolized *P. brevicompactum* collected on the slide of impactor I-1.

The overall capture efficiency and the actual collection efficiency of fungal spores obtained with impactor I-2 confirmed the results found with I-1 (data not shown). While the difference in E_C between the two impactors with the same S/W did not exceed 24%, the collection efficiency curve of impactor I-2 was sharper than that of I-1.

The microscopic count of bacterial and fungal spores on the collection slides revealed a significant number of spore agglomerates (chains and clusters). As an example, Fig. 6 shows the slide with the single spores and agglomerates of *P. brevicompactum* that were collected during 5 min by impactor I-1 ($S/W = 0.099$). It is anticipated that the original percentage of airborne spore aggregates before the impaction was higher and some of them might have deagglomerated (broken up) during impaction. The deagglomeration effect is not an exclusive result of the spore bounce as it is enhanced by considerable shear forces near the impaction slide. Indeed, as the jet-to-plate distance is very low ($S \ll W$), the air streamlines turn sharply from a thin nozzle (throat) of the impactor to even thinner gap between the upper boundary and the impaction plate, in which the air velocity is increased. The ratio of the air velocity in the

circular nozzle of the impactor, V_{NOZZLE}, to one in the gap at the turning point, V_{GAP}, can be expressed as

$$\frac{V_{\mathrm{NOZZLE}}}{V_{\mathrm{GAP}}} = \left(\frac{Q}{\pi W^2/4}\right) \Big/ \left(\frac{Q}{\pi W S}\right) = 4\,\frac{S}{W}. \tag{4}$$

Thus, at $Q = 20\,\mathrm{L\,min^{-1}}$ and for $S/W = 0.033$, the air accelerates approximately from 20 to $50\,\mathrm{m\,s^{-1}}$ at the turning point, creating an increased shear force, which enhances the deagglomeration of spore chains and clusters. Marple (1970) showed that at $S/W < 1$ the flow reattaches to the upper boundary and raises the streamlines off the impaction plate, which may enhance both the deagglomeration and the bounce.

The Reynolds numbers of both impactors, I-1 ($Re \approx 6150$) and I-2 ($Re \approx 3370$) are higher than recommended by the conventional design criteria. Nevertheless, the latter is closer to the upper limit of the recommended range, which makes its collection efficiency curve sharper than that of I-1 (observed in our tests).

The ratio of the single spore count to the propagule count measured for five species (*B. subtilis*, *C. cladosporioides*, *A. versicolor*, *P. brevicompactum*, and *P. melinii*) was found to be within a range of 1.5–2.5. The average percentage of aggregates slightly decreased with the increase of S/W from 0.033 to 0.099. In the field, the break-up efficiency of spore aggregates may differ for different fungal species and environmental conditions.

3.2. Spore enumeration on the slide

The slides obtained during our tests with laboratory-generated spores showed some non-uniformity, especially for larger fungal particles. In our earlier studies, we observed that for circular-jet impactors with $S/W < 1$, the particles tend to create an axisymmetric non-uniform deposit, which we called the "donut" effect (Grinshpun et al., 2002). Similarly non-uniform patterns was observed by Sethi and John (1993) for circular jet impactors. The particles sampled with impactors I-1 ($W = 4.62$ mm) were deposited primarily inside the 4.62 mm diameter circle on the collection surface. The particle surface density appeared to be rather low in the center of the circular deposit area. It was significantly higher at the distance of half-a-radius from the center and then a gradual decrease towards the periphery. In addition, some spores were detected outside of the 4.62 mm circle. Average from 12 tests, 26% of spores were observed outside this "designated" area. One sample of *P. brevicompactum* showed as much as 49%. The deposit spread beyond the 4.62 mm circle was greater, and the deposit non-uniformity inside the circle was more significant, as the aerosol concentration and the percentage of spore aggregates increased. For impactor I-2 that had a smaller nozzle diameter ($W = 2.15$ mm) and consequently smaller deposit area, these effects were less pronounced.

The deposits of *B. subtilis* bacterial spores were more uniform than those of larger fungal spores. The samples of *P. melinii* (the highest spore size among tested) obtained with impactor I-1, showed the highest non-uniformity among the laboratory-aerosolized fungal species with an average number of spores per microscopic field ranging from 4 (center) to 82 (periphery). Nevertheless, the spore count of the entire deposit area and the partial count on 10–20 randomly selected microscopic fields were rather similar (within ±20%). The difference between the "random" and "slice" counts of the laboratory-generated samples did not exceed 9% for *B. subtilis* (minimum) and 27% for *P. melinii* (maximum). Thus, the reading of a "diametric slice" averages up the deposit non-uniformity and seems adequate for the microscopic analysis even if the "donut" effect is significant. The above finding was confirmed by the

Table 2
The inter-sample coefficients of variation of the fungal spore counts obtained by the CyClex BioAerosol Impact Sampler, Cyclex-d cassette, and Micro5 Microcell in two field sites

Field test	Spore type	CV, % ($m = 3$)[a]		
		CyClex	Cyclex-d	Micro5
Site 1	Total	49.2	10.9	35.4
	Aspergillus/*Penicillium*	51.9	12.8	36.8
	Periconia	30.0	19.3	59.2
	Arthrospores	88.6	36.3	60.0
Site 2	Total	2.4	20.9	6.0
	Aspergillus/*Penicillium*	23.7	40.1	14.1
	Periconia	53.2	9.1	41.7

[a] m = number of identical samplers operated in parallel.

analysis of the field samples obtained by the CyClex BioAerosol Impact Sampler. The ratio of "random" to the "slice" fungal spore counts for the six field samples were 0.86, 1.17, 0.58, 0.9, 1.23, and 1.07. The average ratio was approximately 0.97 with the standard deviation of 0.24.

As mentioned above, many available single-stage bioaerosol impactors (e.g., Burkard and Air-O-Cell) produce pronouncedly non-uniform deposits. When the spore samples taken by these impactors are analyzed under the microscope, only partial slide reading is usually performed, as the deposit area is too large to be counted entirely as a routine analysis. Furthermore, the rectangular deposit area on the slide may differ significantly from one sample to another, which is not taken into account by the common slide reading practices. This seems to be a cause of significant variability in the spore enumeration from samples obtained by existing impactors. For circular-jet impactors, the round shape of the deposit represents an additional challenge for the count of the entire deposit area. Therefore, it is important to develop and validate the partial slide reading procedure that would allow generating representative data.

3.3. Variability of the field data

The inter-sample variability was assessed for the three commercially available single-stage circular-jet impactors with a low S/W (CyClex, Cyclex-d, and Micro5). The samples collected by three identical impactors of each type in two mold-problem environments (the total of $3\times3\times2 = 18$ samples) revealed a variety of fungal species. The inter-sample coefficients of variation determined for each set of the three simultaneously operated identical samplers are listed in Table 2. The CVs were calculated based on the random counts of propagules (each spore propagule was counted as one unit). The data represent the total spore counts, as well as some selected genus/group-specific counts for the fungal types, which were found predominant in the tested sites. The variability of the total counts did not exceed 50% for any specific sampler type. Overall, the data obtained in Site 2 showed lower variability than in Site 1 with respect to the total fungal spore count. The same tendency was observed for most of the spore types. Arthrospores demonstrated the highest variability.

The Air-O-Cell cassettes used for the spore collection in the same two field sites demonstrated greater data variability of the total spore count: CV = 56.2% for Site 1 and CV = 28.5% for Site 2 ($m = 3$).

Relatively high variability of spore samples commonly occur in the existing field practices. For example, as has been reported in our earlier paper (Hauck, Grinshpun, Reponen, Reponen, & Willeke, 1997), the CV of indoor fungal spore samples collected with four ($m = 4$) collocated identical filter cassettes ranged from 37% to 61%.

The spore deagglomeration inside impactors and the spore bounce are two factors, which may not only affect the collection efficiency but also contribute to the field data variability. Different species, having different agglomeration/deagglomeration characteristics and bounce properties (which, in turn, depend on the environmental conditions), may be collected with different efficiencies (Trunov et al., 2001). Thus, the total spore count that is affected by the variety of fungal species present in this environment may exhibit considerable variability.

The number of spore aggregates observed in the field samples collected with the CyClex, Cyclex-d, and Micro5 was relatively low. At the same time, independent measurements conducted in these sites with the Air-O-Cell cassettes revealed approximately a two-fold higher percentage of large aggregates, particularly of *Aspergillus*/*Penicillium* and Arthrospores. A low percentage of aggregates, which was observed in the samples obtained by the impactors with low jet-to-plate distance, can be attributed to (i) excessive shear forces that are capable to deaggregate spore agglomerates near the impaction plate, and (ii) considerable bounce effect. In contrast, for conventional impactors with $S/W > 1$, such as the Air-O-Cell, the shear forces are less powerful as $V_{GAP} \ll V_{NOZZLE}$ [see Eq. (4)], and therefore the deagglomeration rate is lower. The particle velocity in the impaction zone ($\sim V_{GAP}$) is much lower in the Air-O-Cell sampler than in the CyClex, Cyclex-d, or Micro5, which results in the smaller bounce rate.

Thus, the decrease in the jet-to-plate distance results in the increase of V_{GAP}, which enhances the spore deaggregation and bounce. The first effect changes the original bioaerosol composition and the second one reduces the number of spores collected inside A_{DEP}. The above may be considered as a limitation of the impactors with low jet-to-plate distances. At the same time, the laboratory study showed a clear benefit of the decreasing of S/W as it helps increasing the collection efficiency of smaller spores. Therefore, the impactors with $S/W \ll 1$ should be designed through the compromise between reducing the cut-off size and minimizing the spore deaggregation and bounce.

The highest difference in the amount of large agglomerates between the Air-O-Cell samples, on the one hand, and the CyClex/Cyclex-d/Micro5 samples, on the other hand, was observed for Arthrospores, which usually occur as chains due to the fragmentation of hyphae. The spore deaggregation and bounce that are pronounced at $S/W \ll 1$ might have contributed to the variability of Arthrospore counts, perhaps to a greater extent than for other fungal types that were less aggregated. This explains the fact that the highest CV was found for Arthrospores.

4. Summary

In this study, we evaluated the single-stage circular-jet impactors with very small jet-to-plate distances ($S/W \ll 1$), which do not meet the conventional design criteria. The laboratory and field data obtained with various test particles (biological and non-biological) demonstrated the feasibility of these impactors for the collection and analysis of airborne spores.

The decrease of the jet-to-plate distance results in a critical decrease of the impactor's cut-off size (d_{50}): from about 2.5 μm of the widely used Air-O-Cell and Burkard samplers to about 1 μm. This makes such an impactor efficient for collecting spores of all fungal species ($\geqslant 1.8$ μm) and even some bacterial species ($\geqslant 1$ μm).

The spore surface density across the circular deposit area was not uniform. The following three slide reading procedures were evaluated: the entire area count, random partial count, and a partial count on the rectangular "diametric slice".

If the jet-to-plate distance is much smaller than the impactor nozzle, excessive shear forces occur in the impaction zone, thus enhancing the spore deaggregation and bounce. The latter two effects seem to have different significance. The efficiencies of capture of solid latex particles reach about 90% suggesting that the role of bounce is not likely to exceed 10%. Thus, the adhesive was evidently effective, at least for a relatively short-term collection. Thus, it appears that deagglomeration may cause more significant concern.

The field evaluation of impactors with $S/W < 10^{-1}$ showed that the coefficient of inter-sample variation was below 50% for the total spore count. The highest variability was observed for Arthrospores, which were more aggregated than other types of fungi.

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References

Adhikari, A., Sen, M. M., Gupta-Bhattacharya, S., & Chanda, S. (2000). Incidence of allergenically significant fungal aerosols in a rural bakery of West Bengal, India. *Mycopathologia, 149*, 35–45.

Aizenberg, V., Reponen, T., Grinshpun, S. A., & Willeke, K. (2000). Performance of Air-O-Cell, Burkard, and Button Samplers for total enumeration of spores. *American Industrial Hygiene Association Journal*, *61*, 855–864.

American Conference of Governmental Industrial Hygienists (1999). *Bioaerosols*: *Assessment and control* (pp. 1–2, 11–15, 11–16, 18–23). ACGIH, Cincinnati, OH.

Bakken, L. R., & Olsen, R. A. (1983). Buoyant densities and dry-matter contents of microorganisms: Conversion of measured iovolume into biomass. *Applied and Environmental Microbiology*, *49*, 1188–1195.

Baron, P., Martinez, A., & Jones, E. (2003). Droplet distortion effect in aerodynamic particle sizing instruments. *Abstracts of the 22nd annual conference of the American association for aerosol research*, Anaheim, CA, October 20–24, 2003.

Bartley, D. L., Martinez, A. B, Baron, P. A., Secker, D. R., & Hirst, E. (2000). Droplet distortion in accelerating flows. *Journal of Aerosol Science*, *31*, 1447–1460.

Burge, H. A. (Ed.) (1995). *Bioaerosols* (pp. 13, 14, 19, 96). Boca Raton, FL: Lewis Publishers.

Cox, C. S., Wathes, C. M. (Eds.) (1995). *Bioaerosols handbook*. Boca Raton, FL: Lewis Publishers/CRC Press.

DeKoster, J. A., & Thorne, P. S. (1995). Bioaerosol concentrations in noncomplaint, complaint, and intervention homes in the Midwest. *American Industrial Hygiene Association Journal*, *56*, 573–580.

Emberlin, J., Newman, T., & Bryant, R. (1995). The incidence of fungal spores in the ambient air and inside homes: Evidence from London. *Aerobiologia*, *11*, 253–258.

Franz, D. R., Parrott, C. D., & Takafuji, E. T. (1997). The U.S. biological warfare and biological defense programs. In: F. R. Sidell, E. T. Takafuji, & D. R. Fraz, (Eds.), *Medical aspects of chemical and biological warfare*. Borden Institute, Walter Reed Army Medical Center, Washington.

Gregory, P. H. (1973). The microbiology of atmosphere (p. 16). Buckinghamshire, UK, Leonard Hill, Aylesbury.

Grinshpun, S. A., Chang, C. W., Nevalainen, A., & Willeke, K. (1994). Inlet characteristics of bioaerosol samplers. *Journal of Aerosol Science*, *25*, 1503–1522.

Grinshpun, S. A., Reponen, T., Willeke, K., Mainelis, G., Górny, R., & Trunov, M. (2002). Collection and enumeration of airborne spores using single-stage impactors. *Abstracts of the seventh international congress on aerobiology*, Château Montebello, Canada, 5–9 August, 2002.

Grinshpun, S. A., Willeke, K., & Kalatoor, S. (1993). General equation for aerosol aspiration by thin-walled sampling probes from calm and moving air. *Atmospheric Environment*, *27A*(9), 1459–1470; *28A* (2) 375.

Hauck, B. C., Grinshpun, S. A., Reponen, A., Reponen, T., & Willeke, K. (1997). Field testing of new aerosol sampling method with a porous curved surface as inlet. *American Industrial Hygiene Association Journal*, *58*, 713–719.

Hill, S. C., Pinnick, R. G., Niles, S., Pan, Y. -L., Holler, S., Chang, R. K., Bottiger, J., Chen, B. T., Orr, C. -S., Feather, G., & Snyder, A. P. (1999). Real-time measurement of fluorescence spectra from single airborne biological particles. *Field Analytical Chemistry and Technology*, *3*, 221–239.

Hinds, W. C. (1999). Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. New York: John Wiley & Sons, Inc.

Horner, W. E., Helbling, A., Salvaggio, J. E., & Lehrer, S. B. (1995). Fungal allergens. *Clinical Microbiology Review*, *8*, 161–179.

Johnson, B., Martin, D. D., & Resnick, I. G. (1994). Efficacy of selected respiratory protective equipment challenged with *Bacillus subtilis* subsp. *niger*. *Applied and Environmental Microbiology*, *60*, 2184–2186.

Levetin, E., Shaughnessy, R., Fisher, E., Ligman, B., & Harrison, J. (1995). Indoor air quality in schools: Exposure to fungal allergens. *Aerobiologia*, *11*, 27–34.

Lin, X., Reponen, T. A., Willeke, K., Grinshpun, S. A., & Foarde, K. (1999). Long-term sampling of airborne bacteria and fungi into a non-evaporative liquid. *Atmospheric Environment*, *33*, 4291–4298.

Madelin, T. M. (1994). Fungal aerosols: A review. *Journal of Aerosol Science*, *25*, 1405–1412.

Marple, V. A. (1970). A fundamental study of inertial impactors. Ph.D. thesis, University of Minnesota, Minneapolis, MN.

Marple, V. A., & Liu, B. Y. H. (1974). Characteristics of laminar jet impactors. *Environmental Science and Technology*, *8*, 648–654.

Marple, V. A., & Willeke, K. (1976a). Impactor design. *Atmospheric Environment*, *12*, 891–896.

Marple, V. A., & Willeke, K. (1976b). Inertial impactors: Theory, design and use. in Liu (Ed.), *Fine particles* (pp. 411–466). New York: Academic Press.

Meldrum, J., O'Rourke, M. K., Boyer-Pfersdorf, P., & Stetzenbach, L. D. (1993). Indoor residential mold concentrations as represented by spore and colony counts. *Proceedings of Indoor Air'93, Vol. 4* (pp. 189–197).

Mitakakis, T. Z., Clift, A., & McGee, P. A. (2001). The effect of local cropping activities and weather on the airborne concentration of allergenic *Alternaria* spores in rural Australia. *Grana*, *40*, 230–239.

Nevalainen, A., Pastuszka, J., Liebhaber, F., & Willeke, K. (1992). Performance of bioaerosol samplers: Collection characteristics and sampler design considerations. *Atmospheric Environment*, *26A*, 531–540.

Rader, D. J., & Marple, V. A. (1985). Effect of ultra-stokesian drag and particle interception on impaction characteristics. *Aerosol Science and Technology*, *4*, 141–156.

Reponen, T. (1995). Aerodynamic diameters and respiratory deposition estimates of viable fungal particles in mold problem dwellings. *Aerosol Science and Technology*, *22*, 11–23.

Reponen, T., Grinshpun, S. A., Conwell, K. L., Wiest, J., & Anderson, M. (2001a). Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition. *Grana*, *40*, 119–125.

Reponen, T., Willeke, K., Grinshpun, S. A., & Nevalainen, A. (2001b). Biological particle sampling. in Willeke, & Baron (Eds.), *Aerosol measurement: Principles, techniques and applications* (2nd ed.), (pp. 751–777). New York: Wiley.

Reponen, T., Willeke, K., Ulevicius, V., Grinshpun, S. A., & Donelly, J. (1997). Techniques for dispersion of microorganisms into air. *Aerosol Science and Technology*, *27*, 405–421.

Sethi, V., & John, W. (1993). Particle impaction patterns from a circular jet. *Aerosol Science and Technology*, *18*, 1–10.

Stewart, S. L., Grinshpun, S. A., Willeke, K., Terzieva, S., & Ulevicius, V. (1995). Effect of impact stress on microbial recovery on an agar surface. *Applied and Environmental Microbiology*, *61*, 1232–1239.

Trunov, M., Trakumas, S., Willeke, K., Grinshpun, S. A., & Reponen, T. (2001). Collection of bioaerosol particles by impaction: Effect of fungal spore agglomeration and bounce. *Aerosol Science and Technology*, *34*, 490–498.

Terzieva, S., Donnelly, J., Ulevicius, V., Grinshpun, S. A, Willeke, K., Stelma, G., & Brenner, K. (1996). Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. *Applied and Environmental Microbiology*, *62*, 2264–2272.