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Use of portable microbial samplers for estimating inhalation exposure to viable biological agents

MAOSHENG YAO AND GEDIMINAS MAINELIS

Department of Environmental Sciences, Rutgers, The State University of New Jersey, 14 College Farm Road, New Brunswick, New Jersey 08901-8551, USA

Portable microbial samplers are being increasingly used to determine the presence of microbial agents in the air; however, their performance characteristics when sampling airborne biological agents are largely unknown. In addition, it is unknown whether these samplers could be used to assess microbial inhalation exposure according to the particle sampling conventions. This research analyzed collection efficiencies of MAS-100, Microflow, SMA MicroPortable, Millipore Air Tester, SAS Super 180, BioCulture, and RCS High Flow portable microbial samplers when sampling six bacterial and fungal species ranging from 0.61 to 3.14 μm in aerodynamic diameter. The efficiencies with which airborne microorganisms were deposited on samplers' collection medium were compared to the particle inhalation and lung deposition convention curves. When sampling fungi, RCS High Flow and SAS Super 180 deposited 80%–90% of airborne spores on agar — highest among investigated samplers. Other samplers showed collection efficiencies of 10%–60%. When collecting bacteria, RCS High Flow and MAS-100 collected 20%–30%, whereas other samplers collected less than 10% of these bioparticles. Comparison of samplers' collection efficiencies with particle inhalation convention curves showed that RCS High Flow and SAS Super 180 could be used to assess inhalation exposure to particles larger than 2.5 μm, such as fungal spores. Performance of RCS High Flow sampler was also reflective of the particle lung deposition pattern when sampling both bacteria and fungi. MAS-100 and SAS Super 180 matched the total deposition curve fairly well when collecting bacterial and fungi species, respectively. For other tested samplers, we observed substantial discrepancies between their performances and particle deposition efficiencies in the lung. The results show that feasibility of applying portable microbial samplers for exposure assessment depends on a particular sampler model and microbial species.
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Introduction

Human exposure to airborne biological agents in residential, occupational, and industrial settings has been shown to cause a variety of negative health effects. Numerous studies have demonstrated that onset of Sick Building Syndrome (SBS) could at least be partially owing to the exposure to the biological agents (Teeuw et al., 1994; Cooley et al., 1998; Walinder et al., 2001; Bholah and Subratty, 2002). Zhiping et al. (1996) have shown that inhalation of bacteria in swine-house dust was correlated with an increase in bronchial responsiveness, decrease in vital capacity, increase in the blood granulocyte concentration and body temperature. High level of exposure to *Sclerotinia sclerotiorum*, an emerging fungal pathogen, was observed during soybean harvesting (Roy and Thorne, 2003). Wouters et al. (2002) indicated that waste collectors exposed to a mixture of bioaerosols showed signs of increased upper airway inflammation and respiratory symptoms compared with controls. Trenter and Walmsley (2003) have indicated that use of ultrasonic dental scaler during the dental operation could release aerosols that may contain dangerous bacteria. Such possibility of aerosol contaminations might also occur during regular dental treatments (MMWR, 1993), thus presenting a serious health concern for the health workers. Gram-negative bacteria in the metal working fluids were identified as possible cause for the outbreak of respiratory disease among 30 workers in an automobile plant (Zacharisen et al., 1998). In addition to these microbial risks in residential and occupational environments, there is an increased threat of bioterrorism which would result in exposure to pathogenic or even lethal microorganisms.

To adequately address these challenges, bioaerosol monitoring and exposure assessment are required. One of the critical steps in such efforts is the selection of a microbiological air sampler. There are many commercially available microbial air samplers utilizing different sampling mechanisms, for example, impaction, impingement, filtration, and electrostatic precipitation. Many of these samplers,

1. Address all correspondence to: Assistant Professor G. Mainelis, Department of Environmental Sciences, Rutgers, The State University of New Jersey, 14 College Farm Road, New Brunswick, New Jersey 08901-8551, USA. Tel: +1 732 932 7166.
E-mail: mainelis@envsci.rutgers.edu

Current address: M. Yao, Department of Chemical Engineering, Environmental Engineering Program, Yale University, New Haven, CT 06511, USA.
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such as Andersen impactor and AGI-30 impinger, require an external vacuum source, which limits their applicability when electricity is not available or user's mobility is desired. In addition, when microorganism concentration is low, high volume sampling devices are needed. Therefore, there is an increasing demand for efficient microbial samplers that are portable, easy to handle, and provide high sampling flow rates.

Over the past several years, a number of new portable microbial samplers have been introduced into the market and several existing ones have been modified. Most of these samplers collect microorganisms directly on culture media and feature sampling flow rates of 100 l/min and higher. Performance of a bioaerosol sampler can be characterized by its physical and biological performances. Physical performance of an aerosol sampler can be described by its collection efficiency curve and cutoff size, or d_{50}. The collection efficiency curve indicates the fraction of airborne particles of certain size that will be removed from the air and deposited onto the collection medium. The cutoff size is the particle size at which the sampler has a collection efficiency of 50%. This parameter can be derived from the experimentally determined sampler's physical collection efficiency curve. Biological performance of the samplers could be characterized by their ability to maintain biological properties of the collected particles. In the case of samplers for culturable organisms, it would be samplers' ability to maintain culturability of collected biological particles. Yao and Mainelis (2006) have investigated the physical collection characteristics of seven portable samplers for culturable bioaerosols when sampling non-biological particles. However, relatively little information is available about their performance when collecting airborne biological particles, which are known to cause a variety of health effects. This study was designed to investigate collection efficiencies of several portable samplers when collecting biological particles.

Also, when performing exposure assessment, it is desired that the samplers perform according to the health-relevant sampling conventions, that is, they adhere to particle inhalation and human lung deposition conventions, thus collecting either respirable, or thoracic, or total inhalable particle fractions. Such sampler's performance allows determining the amount of inhaled aerosols. Health-relevant sampling is widely performed in occupational settings to determine workers' exposure to airborne particles. However, information about the bioaerosol dose and health response is lacking; therefore, it is particularly important not only to determine the presence of bioaerosols in the air but also to estimate the amount of inhaled particles. Application of existing filter samplers for measuring viable and total bioaerosols has been investigated by Kenny et al. (1999) and Aizenberg et al. (2000). The inhalation convention curve recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) defines the desired sampling performance for inhalable particulate matter (IPM) in terms of the fractional collection of particles up to 100 μm in size (ACGIH, 1999). If a bioaerosol sampler is used for exposure assessment, it should conform to sampling criteria, such as inhalation convention curve, in order to provide a better estimate of the amount of the inhaled particles. As bioaerosols may be more detrimental to the human health than non-biological particles, determination of inhaled biological particles is especially important. However, information about the portable bioaerosol samplers' conformity to these sampling criteria is not readily available.

Another way to perform health-relevant bioaerosol sampling could be to determine not just the amount of inhaled particles, but the amount of particles deposited in the human respiratory system. In this case, the sampler's physical performance should mimic either particle deposition in different regions of the respiratory system or overall particle deposition which is defined as the combined deposition of particles in all regions of the respiratory system including head airways, tracheobronchial, and alveolar regions (Hinds, 1999). Certain instruments, such as different collection stages of Andersen impactor have been found to represent particle deposition in various regions of the lung (Rhodes et al., 2001). Based on the International Commission on Radiological Protection deposition model, the total particle deposition efficiency in the respiratory system is a complex function of particle size and the lung geometry (ICRP, 1994). Correlation of total particle deposition efficiency in the lung with the collection efficiency of a bioaerosol sampler for microorganisms of certain size would provide more accurate estimates of actual human exposure to the airborne biological agents. However, very little information is available about whether the portable microbial samplers could be used to determine the amount of culturable bioaerosols inhaled or deposited in the lung.

Therefore, the objectives of this study were to investigate the collection efficiency curves of several portable microbial samplers when sampling biological agents and to determine whether these samplers could be used to determine the amount of microorganisms that are inhaled or deposited in human respiratory system. The second objective was accomplished by comparing the samplers' collection efficiency curves with particle inhalation and deposition conventions for the human lung. Evaluations were performed using three bacterial and three fungal species.

Methods and materials

Test Samplers

This research studied the physical performance and the inhalation conformities of the following portable microbial samplers: MAS-100 (EMD Chemicals, Inc., Gibbstown, NJ, USA) operating at 100 l/min with 50 ml agar, Microflow

(Aquaria srl, Lacchiarella, Italy) operating at 120 l/min with 25 ml agar, BioCulture (AP BUCK Inc., Orlando, FL, USA) operating at 120 l/min with 30 ml agar, SMA MicroPortable (Veltek Associates, Inc., Phoenixville, PA, USA) operating at 141.5 l/min with 25 ml agar, SAS Super 180 (Bioscience International, Inc., Rockville, MD, USA) operating at 180 l/min with 40 ml agar, Millipore Air Tester (Millipore Corp., Billerica, MA, USA) operating at 140 l/min with manufacturer-supplied agar cassette, and RCS High Flow (Biotest Diagnostics Corp., Denville, NJ, USA) operating at 100 l/min with manufacturer-supplied agar strip. All these portable microbial samplers are designed to sample airborne culturable microorganisms for determining presence of microorganisms in the air and for assessing human exposure to the airborne biological agents. Physical characteristics of the samplers used in this study, such as sampling flow rates, number of sampling nozzles, nozzle diameters, jet-to-plate distances, amounts of agar used, throat lengths, S/W ratios (jet-to-plate distance/nozzle diameter), Reynolds numbers as well as the theoretical cutoff sizes (d_{50}), were described or determined in another study (Yao and Mainelis, 2006). The same study also found that experimental d_{50} of investigated portable samplers when sampling non-biological particles ranged from 1.2 μm (RCS High Flow) to 8.8 μm (Microflow).

Test Microorganisms

The performances of the samplers were tested with three bacterial and three fungal species with aerodynamic sizes ranging from 0.6 to 3.1 μm. The bacterial species included vegetative cells of *Bacillus subtilis, Pseudomonas fluorescens,* and *Escherichia coli*. *B. subtilis* is a representative of hardy organisms and also an anthrax surrogate (Hill et al., 1999). *P. fluorescens* bacteria are commonly found in the ambient air environments (Nevalainen 1989, Górny et al., 1999) and are representatives of sensitive bacteria. *E. coli* bacteria have been suggested as one of the standards when testing bioaerosol sampling technologies (Macher, 1997). A recent study implicated airborne spread of *E. coli* O157 during outbreak investigation (Varma et al., 2003). *P. fluorescens* and *E. coli* were found present and posing a health concern in the floodwater following the Katrina disaster in New Orleans, LA (Presley et al., 2006). The tested fungal species included *Cladosporium cladosporioides, Aspergillus versicolor,* and *Penicillium melinii. C. cladosporioides* is one of the dominant fungal species in the air (Cooley et al., 1998). *A. versicolor* and *P. melinii* are believed to belong to the fungi genera that are capable of producing high level of toxic chemicals (Ciegler, 1978). Thus, the selected microorganism species represent sensitive and hardy bacterial species and a surrogate of a biothreat agent as well as toxic fungal species that are commonly found in natural environments.

Stock cultures of *P. fluorescens* (ATCC 13525) and *E. coli* (ATCC 11775) were obtained from American Type Culture Collection Inc. (ATCC, Rockville, MD, USA) and the bacteria were cultured by adding their active cultures into Tryptic Soy Broth (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and keeping the broth inside an incubator shaker (SteadyShake, Amerex Instruments, Inc., Lafayette, CA, USA) at 26°C and 37°C, respectively, for 18 h. *B. subtilis* (ATCC 9372) was cultured by plating its active culture on the Tryptic Soy Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) Petri dish which was kept in the incubator for 18 h at 30°C. Fungal species *C. cladosporioides* (ATCC 11278), *A. versicolor* (ATCC 26644), and *P. melinii* (ATCC 10469) were grown on Malt Extract Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) for 1–2 weeks at room temperature.

Before experiments, *P. fluorescens* and *E. coli* bacteria were separated from broth by centrifugation and then resuspended in autoclaved water. For all other organisms, freshly purified water (Milli-Q, Millipore, Billerica, MA, USA) was added to the agar plates and colonies of the microorganisms were removed from the agar surfaces using an inoculation loop. For all six microorganisms, the initial microbial suspensions were centrifuged at 7000 rpm (BR-4, Jouan) for 7 min. The resulting pellet was resuspended in freshly purified water and centrifuged again. The final pellet of microorganisms from the second centrifugation was suspended in the freshly purified water to be used in the experiments.

Particle Inhalation and Deposition Convention

The inhalable particle fraction is defined as the fraction of particles originally in the volume of air that is inhaled and enter the respiratory system through the nose or mouth (Hinds, 1999). The American Conference of Governmental Industrial Hygienists (ACGIH) recommends the following equation for the inhalable particle fraction (IF) (ACGIH, 1999):

$$\mathrm{IF}(d_a) = 0.5(1 + \exp(-0.06 d_a)) \text{ for } U_0 < 4\,\mathrm{m/s} \quad (1)$$

$$\mathrm{IF}(d_a, U_0) = 0.5(1 + \exp(-0.06 d_a)) + 10^{-5} U_0^{2.75} \times \exp(0.055 d_a) \text{ for } U_0 > 4\,\mathrm{m/s} \quad (2)$$

where d_a is the particle aerodynamic diameter in μm and U_0 is ambient air velocity. Eq. (1) simulates indoor environments with air velocity less than 4 m/s, whereas Eq. (2) simulates outdoor environments with air velocity higher than 4 m/s. The inhalation curve based on these equations defines the desired sampling performance of any inhalable particle sampler.

Once a particle is inhaled, it can deposit inside the respiratory system owing to the impaction, settling, or diffusion mechanism. The International Commission on Radiological Protection developed a model that takes into account the level of activity, gender, breathing rate and frequency, and other parameters (ICRP, 1994). Hinds (1999)

obtained simplified equation for the total particle deposition efficiency in the lung for monodisperse spheres of standard density at standard conditions:

$$\mathrm{DF}(d_a) = \mathrm{IF}(d_a)(0.0587 + \frac{0.911}{1+\exp(4.77+1.485\ln d_a)} + \frac{0.943}{1+\exp(0.508-2.58\ln d_a)}) \quad (3)$$

where $\mathrm{DF}(d_a)$ is the total particle deposition efficiency, $\mathrm{IF}(d_a)$ is inhalation fraction calculated by Eq. (1) or (2), and d_a is the aerodynamic particle size. Eq. (3) calculates the average particle deposition inside the respiratory system for men and women over three exercise levels. The equations above were used to determine the portable samplers' conformity to the particle inhalation and deposition conventions.

Experimental Setup and Collection Efficiency Measurements

The experimental setup as shown in Figure 1 includes bioaerosol generator (Collison nebulizer, BGI Inc., Waltham, MA, USA), and the test system. The microorganisms suspended in water were aerosolized by a Collison nebulizer operated at a flow rate Q_{NEB}, which ranged from 2 to 10 l/min and was adjusted based on the desired aerosol concentration. The resulting aerosols passed through a Po-210 bipolar charger (neutralizer) and were then diluted by an additional filtered airflow, $Q_{\mathrm{DRY}} = 40$ l/min. The used amount of air resulted in air velocity of 0.23 m/s inside the test chamber. Such velocity was selected to simulate the indoor air conditions where air velocity is less than 0.3 m/s (Baldwin and Maynard, 1998; Berry and Froude, 1998). The electrically equilibrated particles entered the bioaerosol sampling chamber where they were subject to collection by the microbial sampler being tested. To obtain a laminar airflow inside the test chamber, we used a flow laminarizer (aluminum honeycomb) as shown in Figure 1.

Before the collection efficiency test, the microorganism aerosol size distribution was measured by an aerodynamic size spectrometer (Aerosizer, Amherst Process Instruments, Inc., Amherst, MA, USA). When performing collection efficiency test, the concentrations of the airborne microorganisms entering and leaving a test sampler, C_{UP} and C_{DOWN}, respectively, were isokinetically measured by an optical particle counter (OPC) (model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA). This instrument operates at a flow rate, $Q_{\mathrm{OPC}} = 1.2$ l/min and measures the equivalent optical diameter of airborne particles in 15 size channels ranging from 0.3 to 20 μm. For all tests, the isokinetic sampling was achieved by adjusting the diameter of the OPCs' probes so that the probes' sampling velocity would match the air velocity upstream and downstream of a test sampler, respectively.

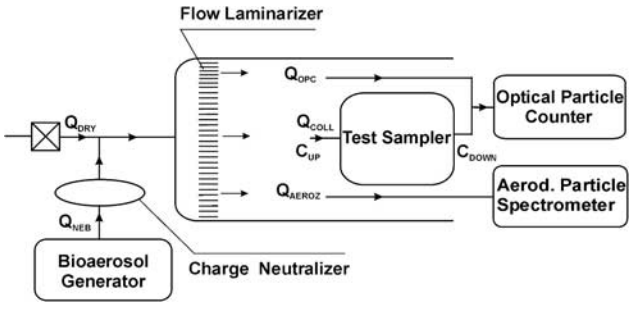

Figure 1. A schematic representation of experimental setup.

The collection efficiency of all seven portable microbial samplers was determined with each test microorganism and each measurement was repeated three times. To account for particle deposition not only on agar but also on air movers positioned behind agar plates, we performed the collection efficiency measurements with and without agar collection plates loaded. The collection efficiency in both cases was calculated using the following equation:

$$E = (1 - \frac{C_{\mathrm{DOWN}}}{C_{\mathrm{UP}}}) \times 100 \quad (4)$$

where E is the collection efficiency for the sampler with or without agar medium plate loaded. The collection efficiency of the sampler with culture media loaded is denoted as $E_{\mathrm{AGAR+FAN}}$, and represents the Overall Collection Efficiency. The collection efficiency without media loaded is denoted as E_{FAN}, the air mover collection efficiency. If we treat the sampler as a two-stage collection system, that is, a fraction of incoming particles is first collected on agar and a fraction of the remaining particles is collected by the air mover, then the Overall Collection Efficiency, $E_{\mathrm{AGAR+FAN}}$, could be described as follows:

$$E_{\mathrm{AGAR+FAN}} = 1 - [(1 - E_{\mathrm{AGAR}})(1 - E_{\mathrm{FAN}})] \quad (5)$$

where E_{AGAR} is the fraction of incoming particles collected on the agar collection medium. By combining Eqs. (4) and (5) we can determine the E_{AGAR} based on the collection efficiency measurements with and without agar medium plate loaded:

$$E_{\mathrm{AGAR}} = (1 - \frac{(1 - E_{\mathrm{AGAR+FAN}})}{(1 - E_{\mathrm{FAN}})}) \quad (6)$$

The E_{AGAR} shows the fraction of airborne particles deposited on agar, and not elsewhere in the sampler, and can be denoted as the Effective Collection Efficiency. The Effective Collection Efficiencies, E_{AGAR}, of each sampler tested with six microorganisms were compared with corresponding particle inhalation and total deposition efficiencies in the human respiratory system. Such a comparison provided basis for determining the inhalation conformity of the samplers studied in this research.

The only exception to the described testing procedure was RCS High Flow sampler because of its different design: it utilizes centrifugation and impaction mechanisms and has no air mover positioned behind the collection medium. Therefore, we did not separately determine the E_{AGAR} and $E_{AGAR+FAN}$. For data analysis purpose, we assumed that the Overall Collection Efficiency of this sampler represents its Effective Collection Efficiency.

Statistical Analysis

Two-way ANOVA test from statistical software developed by the StatPoint, Inc. (Herndon, VA, USA) was used to analyze the Effective Collection Efficiency as a function of two independent variables: microorganism species and sampler model. *P*-values lower than 0.05 indicated statistical significance of a variable.

Results

Bioaerosol Size Distribution

Six species of bacteria and fungi were aerosolized to test the collection efficiencies of portable microbial samplers. These microorganisms ranged from 0.61 to 3.14 μm in mean aerodynamic size as shown in Figure 2. *P. fluorescens* bacteria were the smallest with a mean aerodynamic size of 0.61 μm, and *P. melinii* fungi were the largest particles with a mean aerodynamic size of 3.14 μm. For *E. coli, B. subtilis, C. cladosporioides,* and *A. versicolor*, the mean aerodynamic sizes were 0.78, 0.86, 2.53, and 2.89 μm, respectively. For *C. cladosporioides* and *A. versicolor*, the median aerodynamic size was about 4%–8% larger than the mean aerodynamic, whereas for other microorganisms the mean aerodynamic size was approximately the same as median aerodynamic size. As seen in Figure 2, the size distributions of all microorganisms, except *C. cladosporioides,* feature one distinct peak reflecting their mean aerodynamic size. The size distribution of this particular microorganism had two peaks: the peak associated with smaller size was most likely due to the presence of fungal and mycelia fragments produced during the aerosolization process while the right-hand peak represented spores. The mean aerodynamic size of *C. cladosporioides* was calculated based on the peak with higher aerodynamic size. For other organisms, small peaks to the left of the main peak, as in the case of the *B. subtilis*, is likely the contribution of the culture medium. In the case of fungi, it is likely the contribution of mycelia fragments.

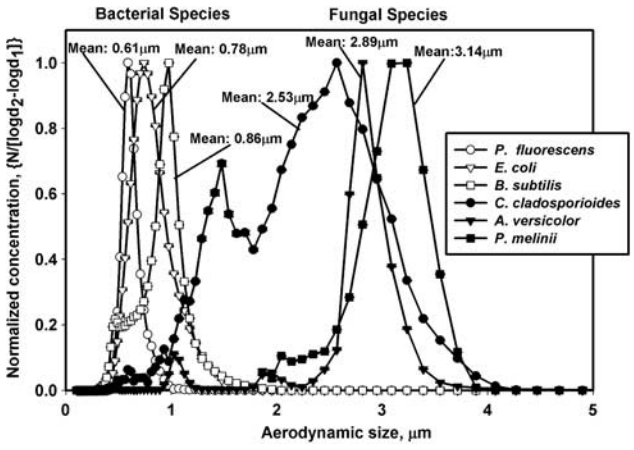

Figure 2. Size distributions of six microorganisms used in this study.

Overall Collection Efficiencies, $E_{AGAR+FAN}$

The portable microbial samplers tested in this study include BioCulture, Microflow, SMA, Millipore Air Tester, MAS-100, RCS High Flow, and SAS Super 180. The Overall Collection Efficiencies of the samplers, $E_{AGAR+FAN}$, were determined using Eq. (4) and are shown in Figure 3. In general, the Overall Collection Efficiency of each sampler increased with increasing microorganism aerodynamic size. When collecting bacterial aerosols of *P. fluorescens, E. coli,* and *B. subtilis*, the Overall Collection Efficiency tended to be rather low for all the samplers tested. For *B. subtilis* aerosols, an anthrax-causing microorganism stimulant, MAS-100, performed best with about 40% Overall Collection Efficiency, whereas RCS High Flow had about 30% Overall Collection Efficiency. For the other five remaining samplers, the $E_{AGAR+FAN}$ was less than 10% for all three bacterial aerosols tested.

When sampling fungal spores of *C. cladosporioides, A. versicolor,* and *P.melinii,* each tested sampler had similar Overall Collection Efficiency for all three fungal species. Microflow had the lowest Overall Collection Efficiency for fungal particles, whereas SAS Super 180 had the highest Overall Collection Efficiency, and efficiency values increased

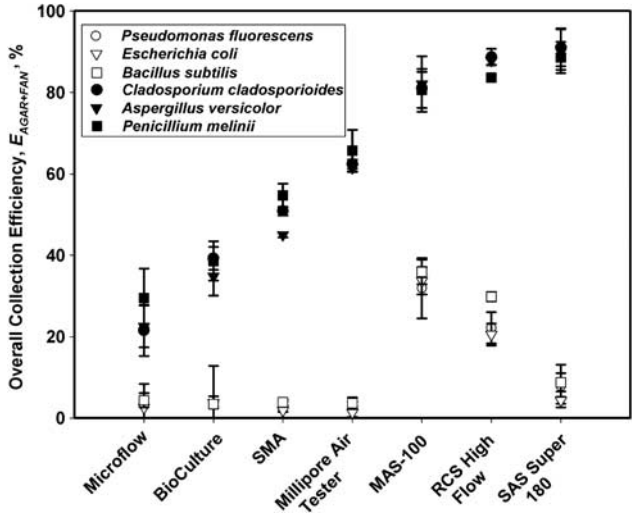

Figure 3. Overall collection efficiencies of seven portable microbial samplers when sampling bacteria and fungi.

in the following order: Microflow, BioCulture, SMA, Millipore Air Tester, MAS-100, RCS High Flow, and SAS Super 180. MAS-100, RCS High Flow, and SAS Super 180 had the Overall Collection Efficiency above 80% when sampling fungal spores larger than 2.5 μm. When sampling fungal spores, the Overall Collection Efficiencies were less than 30% for BioCulture and for Microflow, approximately 50% for SMA, and 70% for Millipore Air Tester. As seen from Figure 3, SAS Super 180 performed best with fungal spores with $E_{\mathrm{AGAR+FAN}}$ of approximately 90%, whereas RCS High Flow and MAS-100 had slightly lower Overall Collection Efficiencies.

Effective Collection Efficiencies, E_{AGAR}

The Effective Collection Efficiencies, that is, the proportion of microorganisms collected onto agar media of the tested samplers are shown in Figure 4. As explained in Materials and methods, the Effective Collection Efficiency of the RCS High Flow is represented by the sampler's Overall Collection Efficiency. As shown in Figure 4, RCS High Flow had the highest Effective Collection Efficiency, approximately 90%, when collecting fungal spores larger than 2.5 μm; SAS Super 180 had the second highest Effective Collection Efficiency — approximately 80%. Millipore Air Tester and MAS-100 had Effective Collection Efficiencies of approximately 60% when sampling fungal spores. For other samplers — SMA, BioCulture, and Microflow, the E_{AGAR} was at 10% level for fungal aerosols tested.

When collecting bacterial aerosols of *P. fluorescens, B. subtilis,* and *E. coli*, RCS High Flow had the highest Effective Collection Efficiency among the samplers tested: about 20%–30%. MAS-100 had Effective Collection Efficiency of approximately 22% when sampling *Bacillus subtilis* and *E. coli* bacteria, whereas this efficiency decreased to below 10% for *P. fluorescens*. The other five samplers — SMA, BioCulture, Microflow, Millipore Air Tester, and SAS Super 180 — had E_{AGAR} of less than 10% when sampling any of the three test bacteria, which are smaller than 1 μm in aerodynamic diameter.

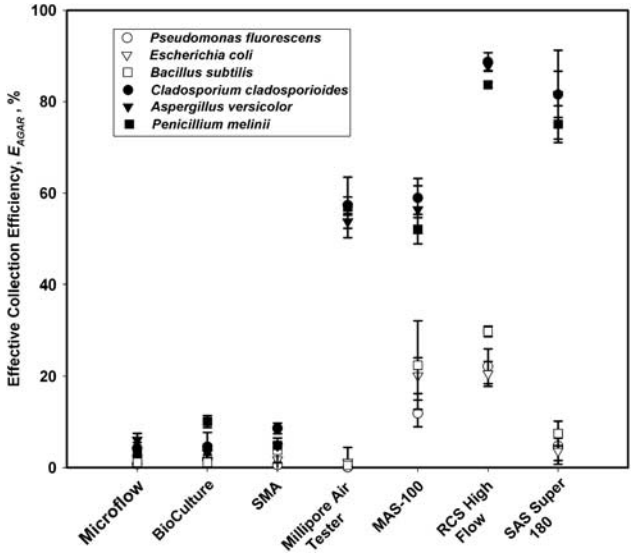

Figure 4. Effective collection efficiencies of seven portable microbial samplers when sampling bacteria and fungi.

The differences in samplers' Effective Collection Efficiencies are due to the differences in their design and operational parameters: dimensionless jet-to-plate distance, throat thickness, number of sampling nozzles, cross-flow parameter, sampling flow rates, and others. The influence of these parameters on microbial samplers' performance has been described in other manuscripts (Marple and Willeke, 1976; Fang et al., 1991; Jurcik and Wang, 1995; Kwon et al., 2002; Yao and Mainelis, 2006). The theoretical d_{50} is directly proportional to the jet velocity. The experimental investigation of d_{50} of the seven portable samplers by Yao and Mainelis (2006) also indicated the contributions of jet-to-plate distance, cross-flow parameter, and other factors.

As only that particular fraction of the airborne microorganisms that deposits directly on collection medium is used for analysis and exposure assessment, the Effective Collection Efficiency should be referred to when selecting a sampler for a particular sampling project. Two-way ANOVA tests showed that Effective Collection Efficiency highly depends on the microorganism size and the sampler type. *P*-values for both variables were less than 0.0001 indicating the statistical significance of both variables.

Comparison of Figures 3 and 4 indicates that high Overall Collection Efficiency does not necessarily predict higher Effective Collection Efficiency. For example, the Overall Collection Efficiency of SMA when sampling *P. melinii* is about 50%, whereas its Effective Collection Efficiency is approximately 10%. Such a difference implies the possibility of the substantial particle losses inside the sampler. Particle losses for the tested samplers are described below.

Sampling losses $(\mathrm{E}_{AGAR+FAN}-\mathrm{E}_{AGAR})$

We calculated each sampler's sampling loss as a difference between the Overall Collection Efficiency, $E_{\mathrm{AGAR+FAN}}$, and Effective Collection Efficiency, E_{AGAR}. The losses indicate the percentage of the microorganisms that were collected not on the collection medium, but elsewhere in the sampler. As shown in Figure 5, the highest sampling losses, approximately 40%–50%, were observed for the SMA sampler, when sampling fungal spores. Microflow, MAS-100, and BioCulture had sampling losses of 20%–30% for fungi and lower losses when sampling bacteria. All other samplers had sampling losses at 10% level. SAS Super 180 had relatively high Effective Collection Efficiency and relatively low sampling loss. The extent of such losses, we believe, depend on the positioning and design of the air mover.

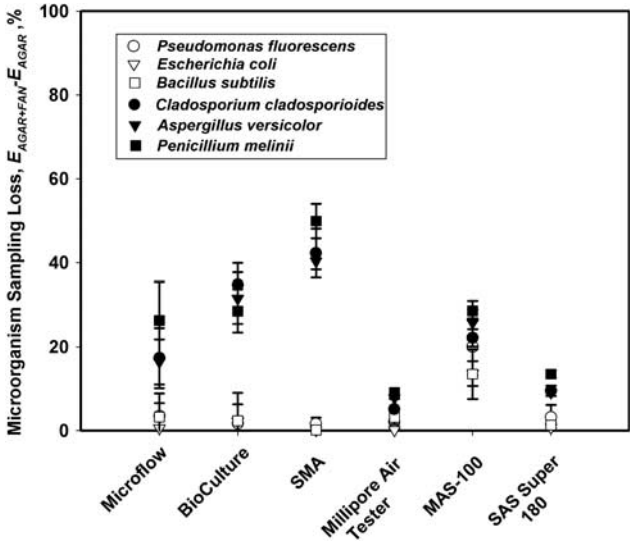

Figure 5. Sampling loss of seven portable microbial samplers when sampling bacteria and fungi.

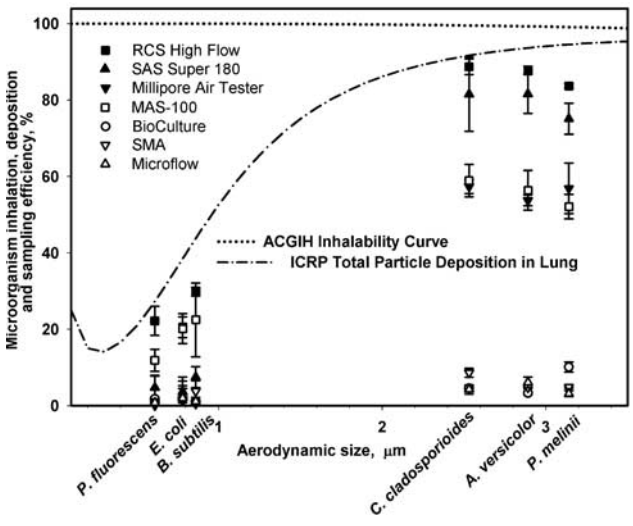

Figure 6. Collection performance of seven portable microbial samplers compared with total particle inhalation and deposition in the lung.

Discussion

The samplers evaluated in this study were developed for determining the presence of biological aerosols in various air environments. If samplers' performances simulate the particle inhalation or deposition efficiency in human lung, they would provide more relevant information related to the microbial exposure. Such information, along with relevant health symptoms, could be used to establish dose–response relationships related to bioaerosol exposure. The inhalable airborne particles can be characterized as the particles originally in the volume of air that are inhaled and enter the nose or mouth (Hinds, 1999). The inhalation convention curve described by Eq. (1) for indoor environments is shown in Figure 6 as a solid line. The fraction of inhalable particles decreases with increasing particle size. From Figure 6 we can see that this fraction is 90% or higher for particles smaller than 3.5 μm, indicating that all six tested microbial particles will be inhaled with efficiency of 90% or higher. As observed from Figure 6, when sampling bacterial species, all tested samplers were found to have substantially lower Effective Collection Efficiencies compared to the inhalation curve, thus significantly undersampling the bacterial species. When sampling larger particles, that is, fungal spores larger than 2.5 μm, RCS High Flow and SAS Super 180 matched the inhalation curve with a difference of less than 10% for RCS High Flow and 15% for SAS Super 180. Millipore Air Tester had a difference of approximately 35% compared with the inhalation curve when sampling fungal particles. For other samplers, the difference was as large as 90% in comparison to the inhalation curve. These results indicate that RCS High Flow and SAS Super 180 are able to reflect the particle inhalation convention fairly well when sampling fungal species, whereas other samplers substantially undersample the fungal particles compared with inhalation convention.

An understanding of particle deposition efficiency in the human respiratory system as a function of particle size provides valuable information needed to adequately assess the health hazard as a result of inhalation of airborne particles. According to the fitted ICRP model for average particle deposition in male and female lungs under three different levels of activity — sitting, light exercise, and heavy exercise, the particle deposition efficiency for particles between 0.1 and 0.25 μm decreases with increasing aerodynamic size (Hinds, 1999). For particles larger than 0.25 μm and smaller than 10 μm, the deposition efficiency increases with increasing particle size (Hinds, 1999). For particles larger than 10 μm, the deposition fraction decreases with increasing particle size (Hinds, 1999). The total deposition of particles in the respiratory system within the investigated particle size range is calculated using Eq. (3) and shown in Figure 6. As seen from the figure, RCS High Flow sampler is able to reflect the particle deposition patterns in the lung when sampling both bacteria and fungi, whereas MAS-100 and SAS Super 180 seem to reflect the particle lung deposition patterns for bacterial and fungi species, respectively. The total deposition fraction is about 35% for the *E. coli* aerosol compared with 22% Effective Collection Efficiency of MAS-100 sampler. There is a difference of less than 10% for RCS High Flow and SAS Super 180 between the total particle deposition and Effective Collection Efficiency for three fungal spores tested ranging from 2.5 to 3.1 μm in aerodynamic size. Effective Collection Efficiencies of other investigated samplers do not seem to reflect the particle deposition pattern in the respiratory system. To address the particle health hazard, both particle inhalability and deposition need to be considered. According

to the ICRP model, the lung deposition efficiency for particles larger than 2.5 μm is above 90%. Performances of RCS High Flow and SAS Super 180 seem to be able to mimic the particle inhalation and deposition patterns for larger particles as observed in our study. Performances of RCS High Flow and MAS-100 seem to be representative of bacterial deposition in the human respiratory system. None of the investigated samplers seems to be able to represent inhalation of bacterial aerosols.

The data presented in Figures 3–6 are based on physical performance of the samplers, that is, particle removal from the air and their deposition on a culture medium. This evaluation does not take into account the biological performance of the samplers, that is, the effect of sampling on the culturability of microorganisms. The extent of such effect would vary depending on the species in question and sampler design.

The obtained data provide basis upon which the portable microbial samplers could be selected for performing a particular health-relevant sampling of culturable biological aerosols. Such sampling could provide more accurate information about the inhaled or deposited amount of culturable biological aerosols and thus contribute to building dose–response relationships relevant to biological aerosols. The results from this study also provide useful information for the bioaerosol detection efforts.

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