A two-stage cyclone using microcentrifuge tubes for personal bioaerosol sampling

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Personal aerosol samplers are widely used to monitor human exposure to airborne materials. For bioaerosols, interest is growing in analyzing samples using molecular and immunological techniques. This paper presents a personal sampler that uses a two-stage cyclone to collect bioaerosols into disposable 1.5 ml Eppendorf-type microcentrifuge tubes. Samples can be processed in the tubes for polymerase chain reaction (PCR) or immunoassays, and the use of multiple stages fractionates aerosol particles by aerodynamic diameter. The sampler was tested using fluorescent microspheres and aerosolized fungal spores. The sampler had first and second stage cut-off diameters of 2.6 μm and 1.6 μm at 2 l min^{-1} (geometric standard deviation, GSD = 1.45 and 1.75), and 1.8 μm and 1 μm at 3.5 l min^{-1} (GSD = 1.42 and 1.55). The sampler aspiration efficiency was ≥ 98% at both flow rates for particles with aerodynamic diameters of 3.1 μm or less. For 6.2 μm particles, the aspiration efficiency was 89% at 2 l min^{-1} and 96% at 3.5 l min^{-1}. At 3.5 l min^{-1}, the sampler collected 92% of aerosolized *Aspergillus versicolor* and *Penicillium chrysogenum* spores inside the two microcentrifuge tubes, with less than 0.4% of the spores collecting on the back-up filter. The design and techniques given here are suitable for personal bioaerosol sampling, and could also be adapted to design larger aerosol samplers for longer-term atmospheric and indoor air quality sampling.

Introduction

Personal aerosol samplers are frequently worn by workers to measure occupational exposure to airborne biological material such as fungi and bacteria. Personal samplers are also used for indoor air quality surveys in homes, schools, and other locations. In the case of bioaerosols, collected materials are frequently identified by culturing samples or by inspecting them with a microscope. However, interest is growing rapidly in analyzing bioaerosols through the use of molecular and immunological techniques such as enzyme-linked immunosorbent assays (ELISA) and polymerase chain reactions (PCR).[1–4] These techniques are faster and less labor-intensive than culture or microscope-based methods, and also provide high levels of sensitivity and specificity.

To simplify sample processing and allow samples to be readily analyzed by PCR and immunoassays, Chen *et al.*[5] developed a single-stage personal aerosol sampler that uses a cyclone design to deposit aerosols into standard Eppendorf-type microcentrifuge tubes. A two-stage version of this sampler has now been developed to collect size-fractionated samples for analysis. In the case of bioaerosols, the two-stage sampler can potentially segregate different microbial species according to their aerodynamic properties. For studies of airborne fungi, the use of a back-up filter allows separation of fungal fragments from intact spores. The purpose of this paper is to describe the design and testing of a two-stage personal aerosol sampler that collects size-fractionated aerosol samples in disposable microcentrifuge tubes. Our discussion will focus on the use of this sampler for fungal bioaerosols.

Fungal and bacterial bioaerosol particle sizes

Bioaerosol particles vary in size depending upon the microorganism, the aerosolization mechanism and the environmental conditions. Meklin *et al.*[6] studied airborne bacteria and fungi in schools and found that airborne particles containing viable bacteria had aerodynamic diameters (d_{ae}) ranging from 0.65 to ≥ 7 μm, with most of the bacterial particles having a d_{ae} between 1.1 and 2.1 μm. Spores of actinomycete bacteria tend to be smaller, with d_{ae} ranging from 0.6 to 1.3 μm.[7] Many fungal spores have a similar aerodynamic size range. Meklin *et al.*[6] found that most of the fungal spores in schools ranged from about 1.1 to 4.7 μm. Reponen *et al.*[8] found comparable results when examining homes and daycare facilities. In a later report, Reponen *et al.*[9] aerosolized individual fungal species and then deagglomerated the spores to break up chains by passing them through an orifice. These authors found that the spores studied had a d_{ae} of 1.8 to 2.8 μm, and that the sizes were fairly characteristic for each species. This study also noted that a variation in the relative humidity from 30 to 90% had only a small effect on d_{ae} and that the aerodynamic diameters were smaller than the physical dimensions of the spores reported in the literature. Gorny *et al.*[10] compared homes with smokers or near steel mills to other homes and found that when particulate levels are higher, bacteria were

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more likely to join agglomerates, while fungal spores occurred primarily as single particles.

The results reviewed above suggest that an aerosol sampler designed to collect fungal spores should have a minimum cutoff diameter of around 1 μm or less, and that a sampler of this type would collect most types of bacterial spores as well. In addition, a first stage with a cutoff diameter of 2 to 3 μm would allow the separation of agglomerates and larger spores from smaller spores.

Fungal bioaerosol collection

Currently, several methods are commonly used to collect bioaerosols. Most exposure assessment studies of fungal bioaerosols have used culture plate impactors. These impactors provide good information about the size distribution of bioaerosols, but they cannot be easily used as personal samplers, and collection times are limited to several minutes at most.[11] Accurate assessments of exposure to airborne fungal materials require the collection of samples for several hours or more because fungal spore concentrations in a building can vary substantially over time.[11]

For personal bioaerosol sampling, filter collection methods are often used and are especially well suited to gravimetric measurements. However, using filters to collect samples for PCR or immunoassays requires that the target aerosol first be extracted from the filter, which can be difficult and time-consuming. In addition, sample recovery from filters varies depending upon the type of filter, the morphology of the target organism and the composition of the sample, which leads to substantial analytical variability.[12] Personal bioaerosol samples also can be collected with liquid impingers, although personal sampling impingers have limited collection times because of fluid evaporation.[13] Samples can be collected for optical inspection using sticky slides or tape strips, but it is difficult to separate samples from the slide or tape adhesive for analysis.

Compared to the methods above, cyclone-based aerosol samplers have several advantages in collecting bioaerosols. Cyclone samplers lend themselves well to extended collection times because they are able to collect relatively large amounts of material. Cyclone samplers can be designed small enough to be used as personal samplers. Cyclone systems also collect samples in a form that makes processing easier, especially if they are designed to accumulate samples in standard laboratory tubes.

Fungal spores and fragments

A growing body of evidence suggests that fungal fragments may be of importance in stimulating allergic reactions in humans.[14] Gorny *et al.*[15] showed with *in vitro* experiments that vibration can stimulate the release of small fungal fragments into the air in much greater abundance than intact spores. Using the same technique, Cho *et al.*[16] found that most fungal fragments have aerodynamic diameters that are less than 1 μm and that these fragments would be expected to deposit more heavily in the lower respiratory tract than intact spores, especially when inhaled by infants. Environmental studies of fungal fragments have been more limited due to the difficultly in identifying and counting fragments, especially small ones. Foto *et al.*[17] collected long-duration air samples in Canadian homes and visually counted spores and fragments. They reported that fungal spore fragments accounted for 6 to 26% of total counts, while hyphal fragments ranged from 4 to 33% of total counts. Green *et al.*[18] collected air samples in an Australian home, counted spores and fragments, and also used a new immunoassay technique to assess allergenicity. They found that the concentration of hyphal fragments was significantly higher than the concentration of spores from certain fungal species, and that 25% of the hyphae expressed detectable allergen.

The results of these studies indicate that fungal fragments need to be included in exposure assessments.[16] Immunological methods are being developed to allow identification and quantification of fungal fragments.[12] However, to accurately assess the presence of fragments, these techniques require very efficient separation of fragments from intact spores. Spore bounce tends to occur with conventional impactors, requiring the use of sticky coatings which limits the possible assay methods.[16] Cyclone samplers are much less prone to spore bounce than conventional impactors, and they are less subject to overloading during longer collections. Operating times can be extended further while maintaining effective separation of spores and fragments by using a two-stage sampler. In this case, the spores are collected primarily on the first stage, with the second stage intercepting any spores penetrating past the first and with fragments collecting on the filter. This allows the collection of larger spore-free fragment samples, which increases the sensitivity of detection during subsequent immunoassay analysis.

Bioaerosol sampler performance

An ideal bioaerosol sampler would draw a nominal volume of air, remove all of the airborne particles within a specified size range, and deposit the aerosol in a collection area without altering the composition, size distribution or biological characteristics of the sample. In reality, sampling losses inevitably occur; particles may not be uniformly drawn into the sampler inlet, or may deposit in the inlet or inside the sampler body rather than in the collection area. The collection process also may reduce the viability of biological organisms or physically damage them. Willeke and Macher[19] describe three forms of sampling efficiency that need to be considered when evaluating a bioaerosol sampler. The first, inlet sampling efficiency (or aspiration efficiency), is a measure of the ability of the sampler to draw a representative unbiased sample of particles from the ambient environment. The second, particle removal efficiency (or collection efficiency), is a measure of the ability of the device to remove particles from the airstream. For a multi-stage sampler, the collection efficiency of each stage is the fraction of a given aerosol entering the collection stage that is retained there. The third form, biological recovery efficiency, refers to the ability of the sampler to collect biological particles without altering their viability, biological activity, physical integrity, or other properties.

For samplers such as cyclones that depend upon inertial effects for aerosol collection, two parameters are generally

used to describe the collection efficiency.[20] The first is the aerodynamic diameter at which the collection efficiency is 50% (called the 50% cut-off diameter, or d_{50}). The second parameter is the geometric standard deviation (GSD) of the collection efficiency curve, which is also called the sharpness. A sharpness of 1.3 to 1.5 is considered sufficient to provide a well-defined particle size cut-off, while a sharpness greater than 2 does not produce a definitive particle separation.[20]

Materials and methods

Aerosol samplers

Three prototypes of the experimental two-stage sampler (Fig. 1) were manufactured and tested. Each prototype used two screw-top 1.5 ml microcentrifuge tubes (#506-624, PGC Scientifics) and ended with a standard 37 mm filter cassette (Model 225-2LF, SKC) used to hold a glass fiber filter (Type A/E, SKC) for microsphere tests, or a gelatin filter (#225-9552, SKC) for spore counting. Two vertical reference samplers[21] were used to assay the test aerosol; each consisted of a sharp-edged 9 mm inner diameter stainless steel tube leading to a 25 mm stainless steel filter holder (#06-100SS, In-Tox Products). The reference samplers also held a glass fiber filter (Type A/E, Gelman Sciences) or a gelatin filter (#12602-25-ALK, Sartorius).

Fluorescent microspheres

Samplers were tested using monodisperse fluorescently-labeled polystyrene microspheres with aerodynamic diameters of 0.5, 1.0, 1.9, and 6.2 μm (Duke Scientific), and 3.1 μm (Polysciences) as described by Chen *et al.*[22] One size of microsphere was used in each experiment. The 0.5 to 3.1 μm microspheres were obtained as a liquid suspension. They were sonicated and diluted sufficiently to ensure that $\geq 98\%$ of the microspheres would be aerosolized as single particles.[23] The 0.5 and 1.0 μm microsphere suspensions were aerosolized using a Model 3076 atomizer (TSI), while the 1.9 and 3.1 μm suspensions were aerosolized using an Upmist medication nebulizer (Model 952, Hospitak). All aerosolized microsphere suspensions were then passed through a diffusion dryer (Model 3062, TSI) and mixed with bipolarly ionized diluent air (Model HPX-1, Electrostatics) to neutralize any accumulated charge on the aerosol.

The 6.2 μm microspheres came as a dry powder and were aerosolized using a Venturi disperser (In-Tox Products) as described previously.[24] The air supply to the disperser was charged using an ionizer (Model HPX-1, Electrostatics) immediately before entering the disperser in order to neutralize any charge resulting from the aerosolization.

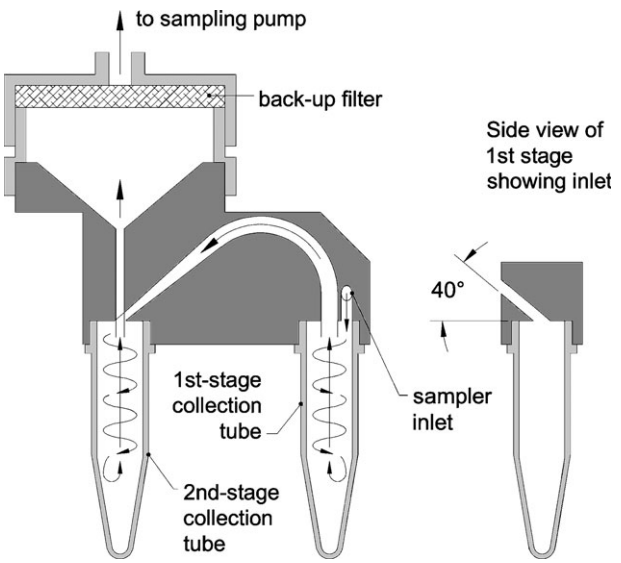

Fig. 1 Personal aerosol sampler using a two-stage cyclone. The sampler is approximately 68 mm wide by 97 mm tall, and weighs 86 g. The first stage inlet has a 2 mm diameter. The channel from the first to the second tube has an initial inner diameter of 3 mm; after bending through a 130° arc, the channel tapers down to 1.3 mm and enters the second tube at a 40° angle. Arrows show the path of the air as it flows through the sampler.

Cultivation of fungal spores

Spores of *Aspergillus versicolor* (ATCC 44408, American Type Culture Collection) and *Penicillium chrysogenum* (NRRL 1951, Northern Regional Research Laboratory, United States Department of Agriculture) were grown in standard sterile unsealed Petri plates containing 5 ml of malt extract agar (2% dextrose, 0.1% peptone, 2% malt extract, 2% agar; Difco, Becton Dickinson). After 7 days of incubation at room temperature, the agar was covered with sporulating colonies and completely dried out. Spores and spore fragments were collected inside a class II biosafety cabinet (Baker Company) by applying 1 g of glass beads (0.45–0.5 mm in diameter; B. Brown Biotech International GmbH) per Petri plate. The lid was placed back on the plate and the plates were gently shaken back and forth. This allowed the beads to roll across the sporulating cultures and the spores and spore fragments to attach to the beads. The beads were then transferred into a sterile 50 ml tube containing 20 ml of distilled water. Spores and fragments were suspended and separated from the beads by briefly shaking the tube and decanting the spore suspension. Spore aggregates were broken up by sonicating the suspension for 15 s at amplitude 6 with the microprobe of a Sonicator 3000 (Misonix). Spore concentrations were determined by hemocytometer count (Bright-Line, American Optical).

Aerosol collection

Samplers were tested in a calm air test chamber described previously.[21] Three prototype samplers and two vertical reference samplers were used during each experiment. The three prototype aerosol samplers were connected to three commercially-available personal air sampling pumps: a Hi Flow Sampler (Model HFS 513A, Gilian), an Airchek Sampler (Model 224-PCXR8, SKC), and an Airchek Sampler (Model 224-PCXR4, SKC). The vertical reference samplers were connected to a central vacuum line through a rotameter (Matheson Gas Products). The air flow through each sampler was calibrated to the same flow rate, either 2 l min^{-1} or 3.5 l min^{-1}, using a flow calibrator (Model 4146, TSI).

During experiments, the test aerosol was aspirated by the three prototype samplers and two vertical reference samplers at the bottom of the calm air chamber. Air was also drawn from the center of the bottom plate by an aerodynamic particle sizer (APS Model 3320, TSI) that was used to monitor the aerosol concentration and size distribution. The inlets of all samplers and the APS were 8 cm above the bottom plate during all tests. The three prototype samplers were rotated among three different test positions so that each sampler was tested an equal number of times in each position and with each sampling pump.

Spectrofluorometry

After aerosol collection was complete, the deposited material was measured in the following six sections: the first stage collection tube (denoted in this paper as the 1st tube); the second stage collection tube (2nd tube); the inlet to the first stage and the top inner surface of the first stage (1st inlet); the channel from the first to the second stage and the top inner surface of the second stage (2nd inlet); the passage from the second stage to the back-up filter including the bottom of the filter plenum (3rd inlet); and the back-up filter (filter). The sum of the aerosol that collected in the 1st, 2nd, and 3rd inlets was defined as the internal deposition. The deposition on the top inner surface of each stage was included with each inlet because it was most practical to rinse these sections together. Each sampler section was rinsed with 5 ml ethyl acetate (Fisher Scientific) to elute the fluorescent dye from the deposited microspheres, except for the back-up filter which was removed and soaked in 10 ml ethyl acetate. Each reference sampler was disassembled, the inlet (denoted as the reference inlet) was rinsed with 5 ml ethyl acetate, and the filter (reference filter) was soaked in 10 ml ethyl acetate. The eluent was filtered through a 13 mm 0.2 μm pore size nylon syringe filter (Fisher Scientific) into a glass cuvette and placed in a spectrofluorometer (Model C-60, Photon Technology International). The fluorescence intensity provided a measure of the relative quantity of microspheres that was collected in each part of the two-stage sampler and the reference sampler.

Aerosol collection efficiency

The collection efficiencies of the first and second stage collection tubes were calculated based on the amount of fluorescent material collected in each tube divided by all of the material collected in that tube and subsequent parts of the sampler. The collection efficiency curve for each stage of the sampler at each flow rate was found by fitting a cumulative lognormal distribution through the collection efficiencies for each particle size using a trust-region non-linear least squares regression (Matlab 7, The Math Works). The fitted lognormal distribution was then used to estimate the d_{50} and the GSD. The goodness of fit of each distribution estimate was evaluated by computing the coefficient of determination (r^2) for each fitted curve. The aspiration efficiency of each prototype sampler was calculated by dividing the sum of the fluorescent material collected in all parts of the sampler by the fluorescence of the reference sample.

Aerosolization and analysis of fungal spores and spore fragments

Fungal spores and spore fragments were aerosolized using a 3-jet Collision nebulizer (BGI) and collected in the same manner as the microspheres described above. For sample analysis, each gelatin filter was dissolved in 1.5 ml centrifuge tubes containing 1 ml of 0.9% NaCl supplemented with 0.2% Tween 20. The tubes were heated for 5 min at 45 °C and vortexed for 1 min to dissolve the gelatin filter and obtain a homogenous spore suspension. Spore suspensions from back-up filters were concentrated 10× by centrifugation for 5 min at 45 °C. Spores in the first or second sampler tubes were suspended by vortexing for 3 min in 1 ml of 0.9% NaCl supplemented with 0.2% Tween 20. During vortexing, the tubes' positions were rotated every 30 s between an upright and upside-down position to ensure the proper suspension of spores accumulated in the lid-proximal portion of the tubes. The three prototype cyclone samplers and two reference samplers were tested in parallel in each of three experiments for each fungal species to determine the overall collection efficiency of the sampler and the fractional deposition of spores in the first and second sampler tubes as well as on the back-up filter. Average spore concentrations in each spore suspension were determined by counting each of four aliquots three times using a hemocytometer. Samplers were rinsed with water and again with reagent alcohol between tests to remove any material that was deposited internally.

Results

Two-stage aerosol sampler design

The design of any cyclone sampler reflects a trade-off between factors including the desired cut-off diameter and collection efficiency sharpness, the desired flow rate, and the amount of pressure drop that is acceptable. Preliminary experiments were conducted using the Chen *et al.*[5] single-stage sampler with a range of inlet diameters and flow rates. After several design iterations, three units of the two-stage personal aerosol sampler (Fig. 1) were manufactured and tested for this project.

The two-stage sampler presented here has a first-stage inlet diameter of 2 mm and a second stage inlet diameter of 1.3 mm. To minimize deposition in the bend leading from the first tube to the second, an outlet orifice diameter of 3 mm was chosen for the first stage. The outlet from the second tube has a 1.5 mm inner diameter. For the first and second stages, the outlet tube (vortex finder) extends 3 mm into the cyclone area. Because of the pressure drop across the sampler, the maximum practical flow rate using a standard commercially-available personal sampling pump was about 3.5 l min^{-1}. For this reason, flow rates of 2 and 3.5 l min^{-1} were selected for experimental evaluation of the two-stage design.

Sampler performance characteristics

The collection efficiency for each tube was determined for air flow rates of 2 l min^{-1} (Fig. 2) and 3.5 l min^{-1} (Fig. 3). At 2 l min^{-1}, the first tube had a 50% cut-off diameter (d_{50}) of 2.6 μm with a sharpness of 1.45, and the second tube had a d_{50}

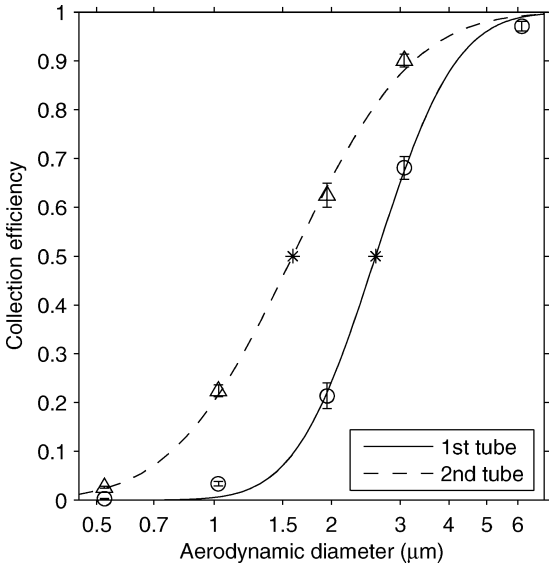

Fig. 2 First and second tube collection efficiencies at 2 l min^{-1}. For each particle size, two experiments were performed with three prototype samplers for a total of six samples. Mean collection efficiencies are plotted for the first tube (○) and second tube (△). Error bars show the standard deviation. A cumulative lognormal distribution was fitted to the results for each tube. For the first tube (—), $d_{50} = 2.6$ μm, GSD = 1.45, and $r^2 = 0.997$. For the second tube (···), $d_{50} = 1.6$ μm, GSD = 1.75, and $r^2 = 0.997$. * denotes the d_{50} for each curve.

of 1.6 μm with a sharpness of 1.75. At 3.5 l min^{-1}, the first tube had a d_{50} of 1.8 μm with a sharpness of 1.42, and the second tube had a d_{50} of 1.0 μm with a sharpness of 1.55. The average

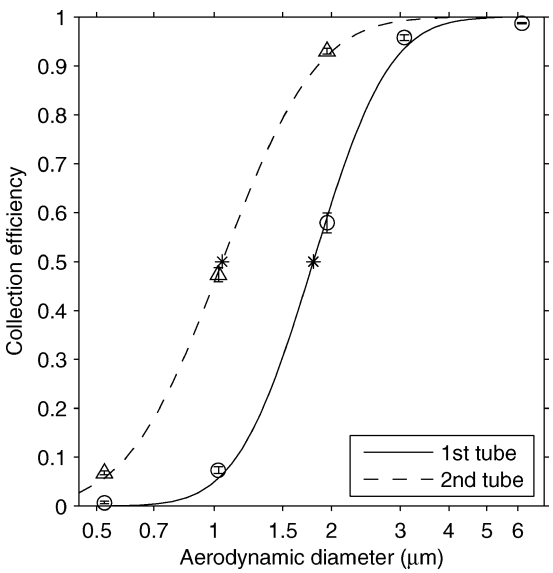

Fig. 3 First and second tube collection efficiencies at 3.5 l min^{-1}. Mean collection efficiencies from six samples are plotted for the first tube (○) and second tube (△). Error bars show the standard deviation. For the first tube (—), $d_{50} = 1.8$ μm, GSD = 1.42, $r^2 = 0.998$. For the second tube (···), $d_{50} = 1.0$ μm, GSD = 1.55, $r^2 = 0.999$. * denotes the d_{50} for each curve.

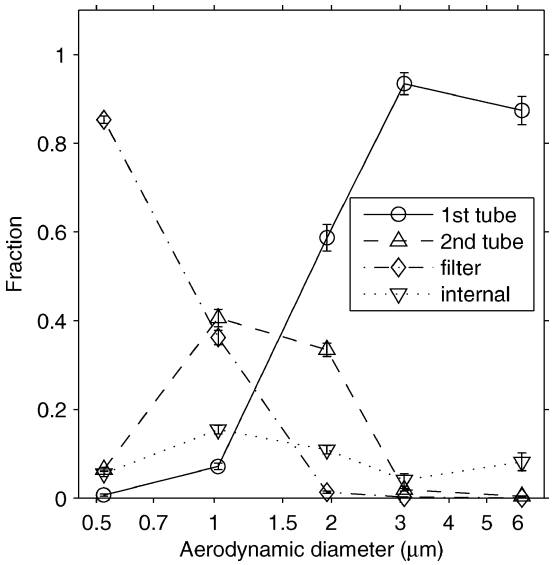

Fig. 4 Distribution of aerosol particles in the sampler at 3.5 l min^{-1}. The lines show the fraction of the collected aerosol found in the first tube (○), second tube (△) back-up filter (◇), and internal deposition (▽). Each data point is the mean of six samples. Error bars show the standard deviation.

pressure drop across the samplers was 17.5 cm H_2O at 2 l min^{-1} and 44.5 cm H_2O at 3.5 l min^{-1}.

As an aerosol passes through the sampler, most of the particles are deposited in the first and second tubes or are collected on the back-up filter. Some particles also are deposited internally around the inlets and outlets, in the curved channel leading from the first to the second tube, and in the path from the second tube to the back-up filter. The amount of material that accumulated in different parts of the sampler is shown for a 3.5 l min^{-1} flow rate (Fig. 4); similar results were obtained for a 2 l min^{-1} flow rate (not shown). At 2 l min^{-1}, the internal deposition reached a maximum of 10% of the total aspirated aerosol for 1.9 μm particles, while at 3.5 l min^{-1} the internal deposition peaked at 15% of the aerosol for 1 μm particles. The aspiration efficiency of the sampler was $\geq 98\%$ at both flow rates for particles with a d_{ae} up to 3.1 μm. For 6.2 μm particles, the aspiration efficiency was 89% at 2 l min^{-1} and 96% at 3.5 l min^{-1}.

Fungal spores

To examine the effectiveness of the two-stage sampler at collecting fungal spores and separating spores from fragments, tests were conducted using aerosolized *A. versicolor* (count median aerodynamic diameter, CMAD, = 2.3 μm, GSD = 1.17) and *P. chrysogenum* (CMAD = 2.3 μm, GSD = 1.17). These species were selected because they are among the most commonly found fungal species in indoor and outdoor environments and because they are known to produce a variety of harmful mycotoxins. At 3.5 l min^{-1}, 84% of the *A. versicolor*, spores were collected in the first tube and 9% in the second tube (Fig. 5). For *P. chrysogenum*, 78% of the spores were retained in the first tube and 14% in the second tube. For both species, spore counts on the back-up filters were $<0.4\%$.

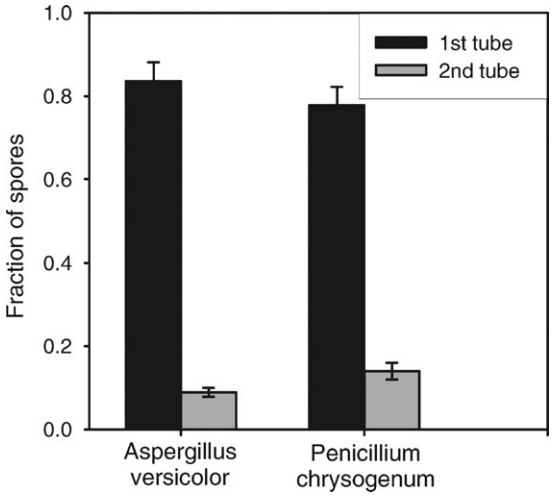

Fig. 5 Collection of fungal spores by the two-stage sampler at 3.5 l min^{-1}. Shown are the mean spore counts found in the three samplers after three experiments. Error bars show the standard deviation.

Discussion

The two-stage sampler that was developed for this project successfully fractionates aerosol samples into useful size ranges for bioaerosol collection. At 3.5 l min^{-1}, the first tube will collect most large fungal spores, large bacteria and agglomerates in the first centrifuge tube, while the second tube will collect most small fungal spores and small bacteria. Very small material such as fungal fragments will be collected primarily by the back-up filter, which also serves to protect the sampling pump from contamination and to prevent the recirculation of material back into the collection area. At 2 l min^{-1}, the sampler would still collect many bacteria and fungal spores, and the higher cut-off diameters may be useful for separating some species or separating individual spores from agglomerates.

The sharpness of the first tube collection efficiency curve is within the 1.3 to 1.5 range for well-defined sampling[20] at the 2 and 3.5 l min^{-1} flow rates. The second tube collection efficiency is almost within this range at 3.5 l min^{-1}; it is not as sharp at 2 l min^{-1} but is still within acceptable limits. It is possible that changes in the second stage outlet diameter or length could improve this performance. Kenny and Gussman showed that changes in the outlet geometry can affect the sharpness, although this effect is difficult to predict.[25]

The collection efficiency of the fungal spores was slightly better than expected based on the aerodynamic diameter of the spores and the results from the microsphere tests. The higher collection efficiency may have occurred because the spores adhere to the microcentrifuge tubes more strongly than the microspheres; during the processing of the spore samples, it was noted that use of the detergent Tween 20 was required to reliably remove the spores from the tube surface for analysis. The sampler was extremely effective at separating fungal spores from fragments; any test spores that bounced past the first tube tended to accumulate in the second tube, and very few intact spores penetrated through to the filter. Thus, this sampler should work very well with immunological assays being developed to quantify fungal fragments. The efficient separation of fragments from spores will also allow animal exposure experiments to determine the relative biological importance of spores and fragments.

Conclusions

The goal of this project was to develop a personal aerosol sampler that uses a two-stage cyclone to collect bioaerosols in disposable centrifuge tubes to improve the speed and accuracy of analysis. The sampler that resulted from this effort is small and lightweight, and is compatible with commercially-available microcentrifuge tubes, sampling pumps and filters. The sampler collects aerosols over a size range suitable for the assessment of bioaerosols containing fungi and bacteria. The sharpness of the collection efficiency curves is acceptable, although further work to improve the second stage sharpness would enhance the sampler performance. Tests conducted using fungal spores verified that the sampler efficiently separates and collects fungal spores and fragments in a manner that is compatible with sample analysis using PCR or immunoassays. Future work will be aimed at improving the sampler design and at developing samplers using larger tubes for longer-term atmospheric and indoor air quality sampling. The samplers will also be used in the development and comparison of PCR and ELISA methods for the analysis of bioaerosols.

Acknowledgements

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References

1 J. Keswani, M. L. Kashon and B. T. Chen, *J. Environ. Monit.*, 2005, **7**, 311–318.
2 C. Vadrot, V. Bex, A. Mouilleseaux, F. Squinazi and J. C. Darbord, *J. Hosp. Infect.*, 2004, **58**, 262–267.
3 Q. Y. Zeng, S. O. Westermark, A. Rasmuson-Lestander and X. R. Wang, *J. Environ. Monit.*, 2006, **8**, 153–160.
4 T. L. Brasel, D. R. Douglas, S. C. Wilson and D. C. Straus, *Appl. Environ. Microbiol.*, 2005, **71**, 114–122.
5 B. T. Chen, G. A. Feather, A. Maynard and C. Y. Rao, *Aerosol Sci. Technol.*, 2004, **38**, 926–937.
6 T. Meklin, T. Reponen, M. Toivola, V. Koponen, T. Husman, A. Hyvarinen and A. Nevalainen, *Atmos. Environ.*, 2002, **36**, 6031–6039.
7 T. A. Reponen, S. V. Gazenko, S. A. Grinshpun, K. Willeke and E. C. Cole, *Appl. Environ. Microbiol.*, 1998, **64**, 3807–3812.
8 T. Reponen, A. Hyvarinen, J. Ruuskanen, T. Raunemaa and A. Nevalainen, *J. Aerosol Sci.*, 1994, **25**, 1595–1603.
9 T. Reponen, K. Willeke, V. Ulevicius, A. Reponen and S. A. Grinshpun, *Atmos. Environ.*, 1996, **30**, 3967–3974.
10 R. L. Gorny, J. Dutkiewicz and E. Krysinska-Traczyk, *Ann. Agric. Environ. Med.*, 1999, **6**, 105–113.

11 A. P. Verhoeff and H. A. Burge, *Ann. Allergy Asthma Immunol.*, 1997, **78**, 544–554.
12 D. Schmechel, R. L. Gorny, J. P. Simpson, T. Reponen, S. A. Grinshpun and D. M. Lewis, *J. Immunol. Methods*, 2003, **283**, 235–245.
13 X. Lin, K. Willeke, V. Ulevicius and S. A. Grinshpun, *Am. Ind. Hyg. Assoc. J.*, 1997, **58**, 480–488.
14 B. J. Green, E. R. Tovey, J. K. Sercombe, F. M. Blachere, D. H. Beezhold and D. Schmechel, *Med. Mycol.*, 2006, DOI: 10.1080/13693780600776308.
15 R. L. Gorny, T. Reponen, K. Willeke, D. Schmechel, E. Robine, M. Boissier and S. A. Grinshpun, *Appl. Environ. Microbiol.*, 2002, **68**, 3522–3531.
16 S.-H. Cho, S.-C. Seo, D. Schmechel, S. A. Grinshpun and T. Reponen, *Atmos. Environ.*, 2005, **39**, 5454–5465.
17 M. Foto, L. L. Vrijmoed, J. D. Miller, K. Ruest, M. Lawton and R. E. Dales, *Indoor Air*, 2005, **15**, 257–266.
18 B. J. Green, J. K. Sercombe and E. R. Tovey, *J. Allergy Clin. Immunol.*, 2005, **115**, 1043–1048.
19 K. Willeke and J. M. Macher, in *Bioaerosols: Assessment and Control*, ed. J. M. Macher, ACGIH, Cincinnati, 1999, pp. 11.11–11.25.
20 J. G. Watson and J. C. Chow, in *Aerosol Measurement Principles Techniques and Applications*, ed. K. Willeke and P. A. Baron, Wiley-Interscience, New York, 1st edn, 1993, pp. 622–639.
21 G. A. Feather and B. T. Chen, *Aerosol Sci. Technol.*, 2003, **37**, 261–270.
22 B. T. Chen, M. D. Hoover, G. J. Newton, S. J. Montano and D. S. Gregory, *Aerosol Sci. Technol.*, 1999, **31**, 24–38.
23 O. G. Raabe, *Am. Ind. Hyg. Assoc. J.*, 1968, **29**, 439–443.
24 Y. S. Cheng, E. B. Barr and H. C. Yeh, *Inhal. Toxicol.*, 1989, **1**, 365–371.
25 L. C. Kenny and R. A. Gussman, *J. Aerosol Sci.*, 2000, **31**, 1407–1420.

