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Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols

Abstract We recently developed an electrostatic precipitator with superhydrophobic surface (EPSS), which collects particles into a 10- to 40-μl water droplet allowing achievement of very high concentration rates (defined as the ratio of particle concentration in the collection liquid vs. the airborne particle concentration per time unit) when sampling airborne bacteria. Here, we analyzed the performance of this sampler when collecting three commonly found fungal spores – *Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor* – under different operating conditions. We also adapted adenosine triphosphate (ATP)-based bioluminescence for the analysis of collection efficiency and the concentration rates. The collection efficiency ranged from 10 to 36% at a sampling flow rate of 10 l/min when the airborne fungal spore concentration was approximately 10^5–10^6 spores/m^3 resulting in concentration rates in the range of 1×10^5–3×10^5/min for a 10-μl droplet. The collection efficiency was inversely proportional to the airborne spore concentration and it increased to above 60% for common ambient spore concentrations, e.g., 10^4–10^5 spores/m^3. The spore concentrations determined by the ATP-based method were not statistically different from those determined by microscopy and allowed us to analyze spore concentrations that were too low to be reliably detected by microscopy.

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Practical Implications

The new electrostatic precipitator with superhydrophobic surface (EPSS) collects airborne fungal spores into small water droplets (10 and 40 μl) allowing achievement of concentration rates that are higher than those of most currently available bioaerosol samplers. Biosamplers with high concentration rates enable detection of low ambient aerial bioaerosol concentrations in various environments, including indoors air, and would be useful for improved exposure assessment. A successful adaptation of the adenosine triphosphate (ATP)-based bioluminescence assay for the quantification of fungal spores from a specific species enables fast sample analysis in laboratory investigations. This rapid assay could be especially useful when investigating the performance of biological samplers as a function of multiple operational parameters.

Introduction

Because of increasing concerns over the negative health effects caused by exposures to bioaerosols (airborne bacteria, fungi, and their metabolites) in various indoor and occupational environments, numerous studies have been conducted to develop more accurate tools to quantify and identify such exposures (Adhikari et al., 2009; Aizenberg et al., 2000; Burge et al., 1989; Gorny et al., 2002; Karol, 1991; Lin and Li, 1998; Reponen et al., 2005).

Furthermore, the continuing threat of exposure to biological warfare agents has spurred the development of new bioaerosol detection systems, and several new bioaerosol samplers have been suggested as candidates for incorporation into such systems (Carlson et al., 2004; Seo, 2007). For liquid-based bioaerosol collectors, one performance metric is the concentration rate, which is a defined as the ratio of particle concentration in the collection liquid vs. the particle concentration in air per unit time. A new wetted-wall bioaerosol cyclone was shown to have concentration rates in the range of 5×10^5–6×10^5/min for 1-μm polystyrene latex particles (PSL) (Hu and McFarland, 2007; Seo, 2007). This cyclone features a continuous liquid outflow at a rate of 1 ml/min. A briefcase-sized

electrostatic precipitator was shown to have a concentration rate of about 1.5×10^4 (Carlson et al., 2004). We previously described an electrostatic precipitator with a superhydrophobic surface (EPSS), which is able to collect airborne particles into very small amounts of liquid (Han and Mainelis, 2008; Han et al., 2010). This sampler achieved concentration rates exceeding 1×10^6/min for two common test bacteria (*Pseudomonas fluorescens* and *Bacillus subtilis* var. *niger*) and non-biological polystyrene latex particles. Such a concentration rate is much higher than that currently achieved by most other bioaerosol samplers. However, the performance of this sampler has not yet been tested with fungal spores.

Fungal spores range from 1.5 to 30 μm in diameter and are generally larger than bacterial cells or their spores (Reponen et al., 2005). Fungal spores are usually hydrophobic and their airborne concentration depends on environmental conditions during their release, e.g., wind speed and turbulence (Levetin, 1995). Therefore, the performance of liquid-based bioaerosol samplers when collecting fungal spores could be different from that when collecting airborne bacteria because of the different physical and biological properties of the particles. As the airborne fungi are responsible for numerous negative health effects (Burge, 2001), it is important to estimate bioaerosol sampler performance when collecting such particles.

When testing the performance of any bioaerosol sampler, the collected bacterial and fungal particles could be analyzed by a variety of methods, including quantitative real-time polymerase chain reaction, direct light and acridine orange epifluorescence microscopy (AOEM), culture-based analysis, and others (An et al., 2006; Seshadri et al., 2009; Williams et al., 2001; Zeng et al., 2004). Among other methods, quantification of adenosine triphosphate (ATP), a basic energy molecule present in all types of living organisms, has been recognized as a convenient and reliable method for estimating total microbial biomass in most environmental samples (Karl, 1980). During the ATP-based bioluminescence reaction, the amount of emitted light is directly proportional to the ATP content or viable biomass. This method has been applied to analyze the presence of viable microorganisms in clean-room facilities (Venkateswaran et al., 2003) and as an indicator of bacterial and fungal activity in cloud water samples (Amato et al., 2007a,b). It has also been suggested as one of the methods to monitor the microbiological contamination of spacecrafts/instruments (Olsson-Francis and Cockell, 2010).

The output from the ATP bioluminescence assay is recorded as relative luminescence units (RLU), and RLU varies depending on the species-specific ATP content. In general, it is recognized that bacterial endospores have a lower ATP content (10^{-21} mol ATP per spore) compared to vegetative cells (10^{-17} mol ATP per cell) (Kodaka et al., 1996). Fungal spores, however, have a higher ATP content compared to that of vegetative bacterial cells (Rakotonirainy et al., 2003). As the output of the ATP bioluminescence assay depends on the microorganism species, the studies mentioned previously used the assay to estimate the overall presence of viable microorganisms. However, when species-specific calibration curves relating luminescence intensity with the cell concentration are prepared, one could use this method to quantify pure cultures. We successfully used this approach to rapidly quantify bacteria when analyzing the collection efficiency of a bioaerosol sampler (Seshadri et al., 2009). This method, however, has not been applied for testing the effectiveness of biosampler collection of fungal spores. As the ATP assay is considered particularly applicable for monitoring samples from environments with extremely low microbial burden (Venkateswaran et al., 2003) and is a sensitive and time-saving method for detecting viable fungal spores (Rakotonirainy et al., 2003), we also investigated the applicability of the method to test the performance of the EPSS when sampling fungal spores.

Thus, the purpose of this study was twofold: (i) to adapt and calibrate the ATP-based bioluminescence method for testing aerosol samplers with pure fungal cultures and (ii) to investigate the performance of the electrostatic precipitator with superhydrophobic surface (EPSS) when collecting fungal bioaerosols using the ATP method. The EPSS was challenged with three commonly found fungal spores – *Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor* and its performance was tested as the function of the collecting water droplet size (10 and 40 μl), the airborne fungal concentration (10^4–10^7 spores/m^3), and the sampling time (10, 30, and 60 min). In addition, we also investigated conditions that resulted in the optimum performance of the ATP method for the quantification of specific fungi, such as the duration of fungal growth prior to harvesting.

Materials and methods

Biological test particles

The fungal species used in this study, *C. cladosporioides* (ATCC 5899; American Type Culture Collection, Manassas, VA, USA), *P. melinii* (ATCC 10469), and *A. versicolor* (ATCC 26644), are common in indoor and outdoor environments (Horner et al., 1995; Madelin, 1994). All cultures were plated onto Sabouraud dextrose agar (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and incubated at room temperature (approximately 26°C) for seven days. During the optimization of the ATP method, the incubation time varied from 5 to 21 days. After incubation, about 3 ml of sterile deionized water was

added to each plate and the spores were gently harvested from mycelium using a spreader. The volume of the resulting spore suspension was increased to 50 ml, and the spores were harvested by centrifugation at 5050 g for 5 min at 4°C (BR4; Jouan, Winchester, VA, USA) and then washed five times with sterile deionized water under the same conditions as described previously (Yao and Mainelis, 2006). The resulting spore pellet was resuspended in sterile deionized water and then diluted 10-fold several times to achieve the target airborne concentration of approximately 10^5–10^6 spores/m^3 for most of the experiments, as determined by an Aerodynamic Particle Sizer (APS, Model 3321; TSI Inc., Shoreview, MN, USA). The final spore suspension was examined under a microscope to verify that the vast majority (>95%) of particles were spores with very few fragments of mycelium.

Sampler, experimental setup, and sample collection

The EPSS was described in detail in our earlier publications (Han and Mainelis, 2008; Han et al., 2010). Briefly, the EPSS is a novel electrostatic precipitator with the shape of a closed half-pipe and is composed of two main parts: the curved and conductive top surface that serves as a ground electrode and the flat bottom plate that houses a narrow collection electrode covered by a superhydrophobic substance (HIREC-1450; NTT Corporation Inc., Tokyo, Japan) connected to high voltage. This electrode is positioned slightly below (0.3–0.5 mm) the surface of the plate for the improved guidance of the collecting droplet. A 3.2-mm-wide collection electrode was used for 40-μl droplets, while a 2.1-mm electrode was used for 10-μl droplets. The entire device was positioned at a slight angle (5°) relative to horizontal. During sampler operation, electrically charged particles are electrostatically deposited on the collection electrode. At the end of a sampling period, a droplet is introduced at the top of the collection chamber and it rolls down under gravity removing the collected particles.

The schematic of the experimental setup is shown in Figure 1. For each test, a 30-ml suspension of fresh fungal spores in sterile deionized water was aerosolized using a Collison nebulizer (BGI Inc., Waltham, MA, USA), operated at a flow rate, Q_A, of 4 l/min. After aerosolization, the spore-laden air was diluted with a HEPA-filtered air flow, Q_D, of 36 l/min. The resulting aerosol stream of 40 l/min was passed through a 2-mCi Po-210 charge neutralizer. The electrically neutralized bioaerosols then passed through a 0.035-m duct housing a vertically oriented ionizer (Wein Products Inc., Los Angeles, CA, USA), which imparted positive charge on the particles (V_{CHARGE} = 12 V/50 mA) as described previously (Han and Mainelis, 2008; Han et al., 2010). A stable DC power supply (BK Precision, Yorba Linda, CA, USA) provided power to the ionizer. The positively charged bioaerosol particles passed through a flow straightener and then entered a cylindrical test chamber (approximately 0.10 m in diameter and 0.25 m in length), where they were aspirated into the EPSS, operated at a sampling flow rate, Q_S, 10 l/min. After a sampling time, t, of 10–60 min, the fungal particles deposited on the collecting electrode were removed by a water droplet rolling along the length of the electrode under the force of gravity. The water droplet was collected in a vial. Another stable high-voltage DC power supply (Bertan Associates, Inc., Valhalla, NY, USA) provided collecting voltage (V_{EPSS} = −7kV) to the EPSS. The entire experimental setup was housed inside a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN, USA).

Development of bioluminescence method for the analysis of fungal concentrations

Effect of culture age. The bioluminescence intensity of a sample is proportional to its ATP content, which, in turn, is proportional to the concentration of biological particles in a sample (Eydal and Pedersen, 2007). When applying this method for fungi, we followed procedures

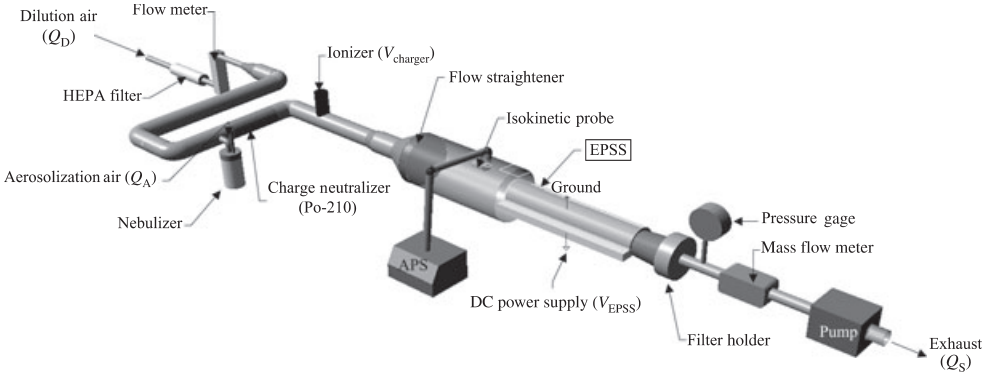

Fig. 1 Schematic diagram of the experimental setup. The isokinetic probe upstream of the EPSS was used to determine the bioaerosol reference concentration.

we previously developed for the quantification of bacteria (Seshadri et al., 2009). However, as the growth time of fungal cultures may affect the intensity of bioluminescence, the first set of experiments was performed with fungal cultures of different ages: 5, 7, 9, 12, 14, and 21 days to determine the optimum growth time. From each 30-ml suspension containing the test fungi, triplicate 200-μl aliquots were removed and divided equally between two 1.5-ml centrifuge tubes: 100 μl for the ATP analysis and 100 μl for analysis by AOEM. For ATP analysis, the selected volume of fungal suspension (100 μl) was combined with an equal volume of Bactiter-Glo reagent (Promega Corp., Madison, WI, USA); the contents were briefly vortexed and then left at room temperature for 9 min. The luminescence intensity of the resulting suspension was measured by a luminometer (model 20/20n; Turner Biosystems Inc., Sunnyvale, CA, USA) and recorded as RLU. The luminescence intensity was correlated with the total number of fungal spores in 100 μl determined by AOEM ($N_{\text{sample, AOEM}}$) as described below. The ratio of luminescence intensity (RLU) normalized to the $N_{\text{sample, AOEM}}$ and plotted as a function of culture age for each fungal species is shown in Figure 2. The highest ratios for *C. cladosporioides*, *P. melinii*, and *A. versicolor* were 7.7 ± 0.4, 5.9 ± 1.0, and 38.8 ± 7.0 RLU/spore, respectively, and they were obtained with 7-day-old cultures. Based on these results, a culturing period of 7 days was chosen as optimal for all the subsequent experiments.

Preparation of calibration curves. It is evident from Figure 2 that different fungal species produce luminescence signals of different strength. Thus, separate calibration curves were prepared for each test species.

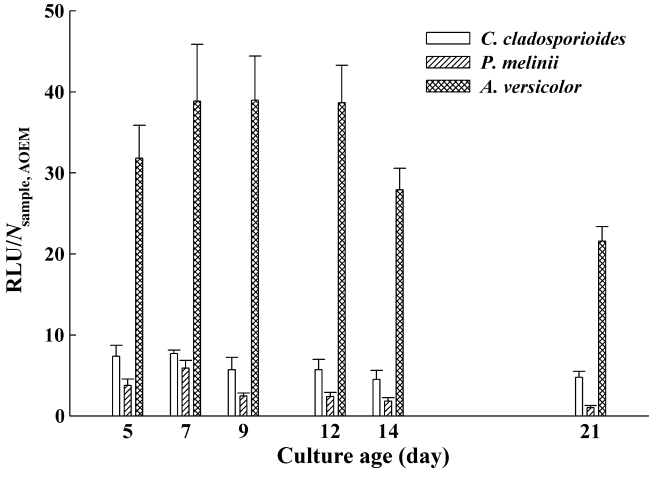

Fig. 2 Ratio of luminescence intensity (RLU) normalized to the total number of spores ($N_{\text{sample, AOEM}}$) for each liquid fungal spore sample (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) as a function of culture age (5, 7, 9, 12, 14, and 21 days). The error bars represent the standard deviation from three repeats

In addition, our earlier research (Seshadri et al., 2009) indicated that to improve the accuracy of the method, the calibration curves should be study-specific, i.e., they should be prepared using the same sampling protocol that will be used to collect and analyze actual samples. The calibration curve for each fungal species represents a relationship between the RLU reading from the luminometer and the total spore counts obtained by AOEM.

Thus, when preparing the calibration curves, test fungi were aerosolized and spores were collected by EPSS into 40-μl droplets using the same procedures that were later used to determine the collection efficiency of the EPSS. The 40-μl samples collected by the EPSS were increased in volume to 200 μl by adding sterile and purified water and then diluted in a series of 10-fold serial dilutions ranging from 10^0 to 10^{-2}. A 180-μl aliquot from each dilution was divided equally between two 1.5-ml centrifuge tubes: 90 μl for the ATP analysis and 90 μl for AOEM analysis. Each sample for the ATP analysis (90 μl) was combined with an equal volume of Bactiter-Glo reagent and its luminescence intensity was determined. The volume of the second 90-μl aliquot was increased to 1 ml, and the total concentration of fungi was determined by AOEM.

The calibration curves were obtained by plotting number of spore per 20 μl (half of the EPSS droplet volume) determined via AOEM vs. the RLU values obtained for 20 μl (Figure 3). The coefficient of determination, $R^2 = 0.999$, was obtained for all three species. The number of spores in an unknown sample could then be determined based on the RLU value using the equations shown in Figure 3:

$$N_{\text{sample, ATP}} = f(\text{RLU}) \tag{1}$$

where $N_{\text{sample, ATP}}$ is the number of spores in a sample based on the ATP measurement. For any given spore

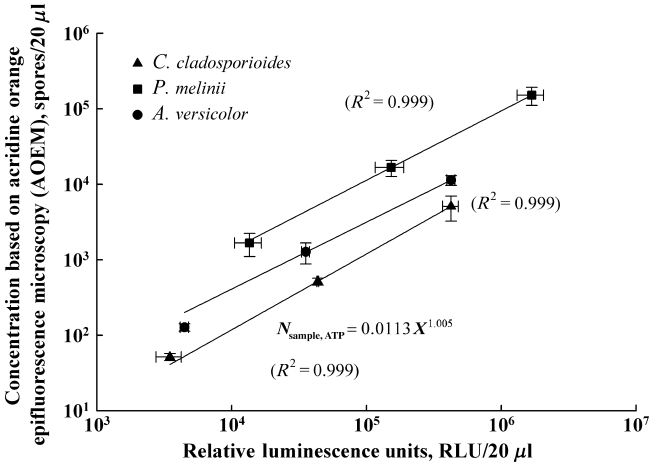

Fig. 3 Standard curves of *Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor* based on the relationship between the RLU values and the fungal concentration as determined by the epifluorescence microscopy

concentration, the highest luminescence intensity was observed from the *C. cladosporioides* spores and the lowest from the *P. melinii* spores. If the RLU values for *P. melinii* were set to 1, then intensity ratios for *C. cladosporioides* and *A. versicolor* would be approximately 11.0 and 4, respectively, for the same concentration. The reliable quantification ranges allowed by the ATP method and AOEM were 50–5×10^3 spores/20 μl for *C. cladosporioides*, 1.6×10^3–1.5×10^5 spores/20 μl for *P. melinii*, and 1.3×10^2–1.1×10^4 spores/20 μl for *A. versicolor*. Quantification of lower spore concentrations was unreliable because of the properties of individual species (weak signal) and the background luminosity. All the ATP measurements were performed with water diluents and were adjusted for average background values for water (RLU = 1500).

When calculating the collection efficiency of the EPSS, the number of collected fungal spores was determined using the corresponding RLU values and equations shown in Figure 3. As could be seen from the Figure, the calibration equations are quite different for each fungal species, and, thus, quantification of spores is applicable for pure cultures and not for mixed bioaerosol samples. On the other hand, as the ATP-based bioluminescence method allows rapidly quantifying spores in pure cultures, it could be a suitable and time-saving alternative to microscopic counting, especially for laboratory investigations.

Quantification of fungal spores collected by the EPSS

The number of fungal spores collected by the EPSS was determined by both AOEM (An et al., 2006), using an Axioskop Imager A1 microscope (Carl Zeiss Micro-Imaging Inc., Thornwood, NY, USA), and the ATP bioluminescence method. The volume of the sample collected by the EPSS (10 or 40 μl) was increased to 200 μl with sterile purified water and was then divided into two equal 100-μl parts: one for ATP analysis and the other for AOEM analysis. Each portion was placed into a 1.5- ml centrifuge tube.

For ATP analysis, the first 100-μl aliquot was combined with an equal volume of Bactier-Glo reagent (Promega Corp.) and luminescence intensity was determined as described previously.

For AOEM analysis, the volume of the second 100-μl aliquot was increased to 1 ml by adding sterile purified water and the sample was serially diluted in 10-fold dilutions with sterilized water to achieve a concentration that could be counted by AOEM. Each slide used for microscopy was prepared by filtering a 1-ml aliquot of the selected dilution through a 25-mm 0.22-μm porosity black polycarbonate filter (Fisher Scientific, Suwannee, GA, USA) and then staining it with 1 ml of 100 μg/ml acridine orange solution (Biotium, Inc., Hayward, CA, USA) for 15 min. After staining, the filter was mounted on a glass microscope slide with immersion oil and a cover slip. At least 40 randomly chosen microscope fields were counted using a 40× objective. The total spore number in each sample, $N_{\text{sample, AOEM}}$, was calculated as follows:

$$N_{\text{sample, AOEM}} = N \times X \times D_{10-\text{fold}}. \quad (2)$$

Here, N is the average spore count in each view field of the microscope, X is the number of fields for the entire filter (X = 980 for Axioskop A1), and $D_{10\text{-fold}}$ is the dilution factor.

Determination of the collection efficiency

The observed efficiency with which spores entering the EPSS were removed from the air stream inside the EPSS was above 90% as determined in our preliminary tests for our previous study (Han and Mainelis, 2008). This result was similar to our observation of similar-sized PSL particles. As the ratio of the number of spores in a droplet(s) vs. the number of spores drawn into the EPSS during a collection period (reference concentration) represents the overall collection efficiency of the EPSS, we used it as our primary experimental metric.

The majority of particles deposited on the collection electrode of the EPSS are removed by the first-introduced water droplet, as shown in our earlier work with bacteria and polystyrene particles (Han and Mainelis, 2008; Han et al., 2010). Our preliminary experiments with fungal spores also indicated that the majority of removable spores were removed by the first droplet, while the second droplet usually contained 4–5%, and the third droplet contained 1–2% of removable spores. As the primary goal of the sampler was to achieve a high concentration rate, the collection efficiency and the concentration rate were calculated based on the first collecting water droplet.

To determine the reference concentration, fungal spores in the test chamber were isokinetically collected on a 47-mm membrane filter (Pall Inc., East Hills, NY, USA) and simultaneously counted with an aerodynamic particle sizer (APS) (model 3320; TSI Inc.) using an isokinetic probe. Spores collected on a filter were extracted into sterile deionized water using the procedure described elsewhere (Wang et al., 2001). The number of spores in the resulting suspension was determined by AOEM and compared with the concentration measured by the APS. It was found that the two number concentrations (APS reading vs. reference filter) agreed within 9.8%, which was deemed an acceptable agreement given the inherent uncertainty in microorganism counting by microscopy (the standard deviation is usually approximately 20%). Thus, to simplify our experimental procedures, the reference spore number was provided by the APS and the collection efficiency of the EPSS, η, was determined as:

$$\eta = \frac{N_{\text{sample}}}{N_{\text{reference}}} = \frac{N_{\text{sample,AOEM}}}{R_{\text{APS}} \times 1000 \times Q_{\text{s}} \times t} \text{ or}$$
$$\eta = \frac{N_{\text{sample,ATP}}}{R_{\text{APS}} \times 1000 \times Q_{\text{s}} \times t}. \quad (3)$$

Here, N_{sample} is the number of spores collected in a water droplet (10 or 40 μl) as determined either by the AOEM ($N_{\text{sample, AOEM}}$) or the ATP ($N_{\text{sample, ATP}}$) method; $N_{\text{reference}}$ is the reference spore number measured by the APS, R_{APS} is the average spore concentration (#/cm^3) measured by the APS every 20 s for a 10-min sampling time (every 60 s for 30 min and every 120 s for 60-min sampling time), Q_{s} (l/min) is the sampling flow rate of the EPSS, and t (min) is the sampling time. The concentration rate, which is a ratio of particle concentration in the collection liquid vs. the airborne particle concentration per time unit, can also be expressed as follows (Han and Mainelis, 2008):

$$R_C = \frac{Q_{\text{s}}}{V}\eta, \text{ min}^{-1} \quad (4)$$

where V is the droplet volume (l).

The effect of the sample analysis method (AOEM vs. ATP) for different fungi and different droplet sizes was analyzed using ANOVA.

Results and discussion

Figure 4 shows the representative number concentration and particle size distributions of airborne *C. cladosporioides, P. melinii*, and *A. versicolor* spores measured by the APS averaged over 10-min period. The mode diameter for *C. cladosporioides*, *P. melinii*, and *A. versicolor* are approximately 2.0, 2.1, and 1.7 μm, respectively.

Figures 5 and 6 show the collection efficiency and the resulting concentration rate of the EPSS, respectively, when sampling fungal spores at 10 l/min and using water droplets of 10 and 40 μl to remove the collected particles. The collection efficiency, η, was determined for airborne spore concentrations of approximately 10^5–10^6 spores/m^3 which, as was determined in the preliminary experiments, could be comfortably counted via microscopy without dilution. The sampling time in these tests was 10 min, and the number of collected spores was determined by the AOEM and ATP methods. As could be seen from Figure 5, the collection efficiencies of the EPSS measured by the ATP and AOEM methods were 21 ± 2.3% and 21 ± 0.4% for *C. cladosporioides*, 36 ± 2.4% and 36 ± 2.0% for *P. melinii,* and 25 ± 2.8% and 23 ± 2.7% for *A. versicolor* when using 40-μl collection droplets. For each analysis method and for each spore type, the collection efficiency was lower with the smaller water droplet (10 μl) compared to the larger droplet (40 μl). The highest decrease, approximately 50%, was observed for *C. cladosporioides*. For the other two fungi, the decrease was 20–30%. A similar dependence of the collection efficiency on the size of the collecting droplet was observed when testing with PSL particles of different sizes. The decrease was attributed to a less efficient removal of particles deposited on the collection electrode by a smaller droplet (Han and Mainelis, 2008). Overall, the collection efficiency for *C. cladosporioides* was the lowest among the three species tested. There was very good agreement in the collection efficiencies determined using the AOEM and ATP methods for all test conditions (three types of test particles and two droplet sizes), and the differences were not statistically significant ($P > 0.05$).

Figure 6 shows the concentration rates based on the collection efficiency data presented in Figure 5. As could be seen, the concentration rates for *C. cladosporioides*, *P. melinii*, and *A. versicolor* reached approximately 5×10^4, 9×10^4, and 8×10^4/min, respectively, for the 40-μl water droplet, while the concentration rates were 1×10^5, 3×10^5, and 2×10^5/min, respectively, for the 10-μl water droplet. These concentration rates were lower than values observed for bacteria

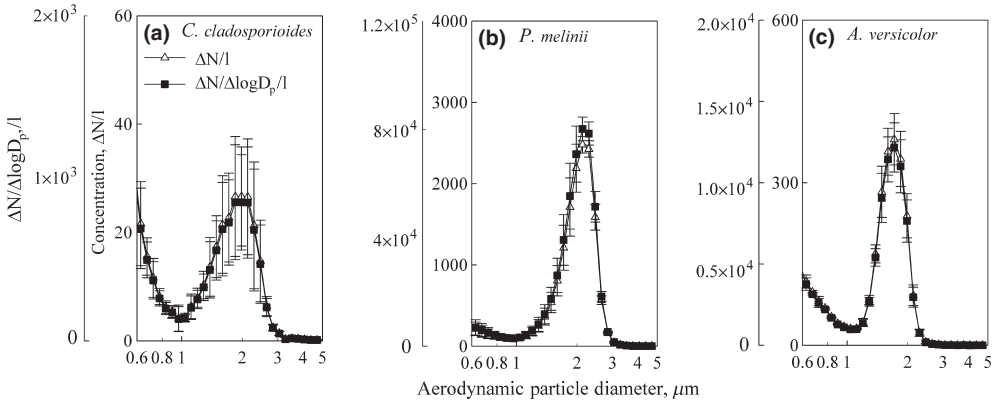

Fig. 4 Particle size distributions according to number concentration (ΔN/l) and normalized number concentration (ΔN/ΔlogD$_p$, /l) for three fungal species: (a) *Cladosporium cladosporioides*, (b) *Penicillium melinii*, and (c) *Aspergillus versicolor*

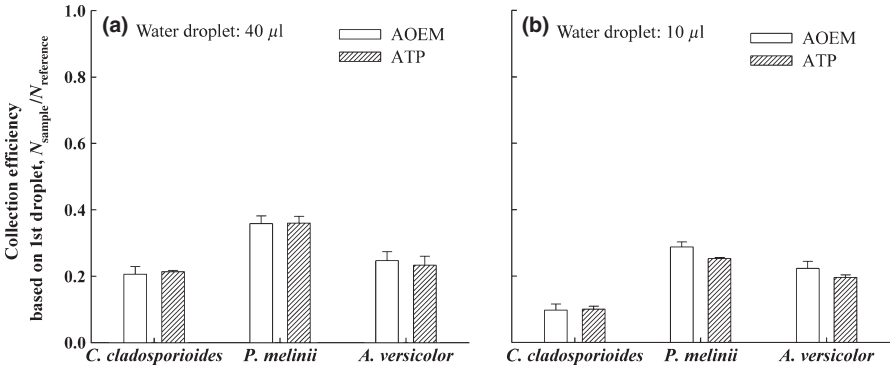

Fig. 5 Collection efficiency of the EPSS when sampling fungal spores (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) based on the 1st collecting water droplet (40 and 10 μl) at 10 l/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage. The error bars represent the standard deviation from three repeats

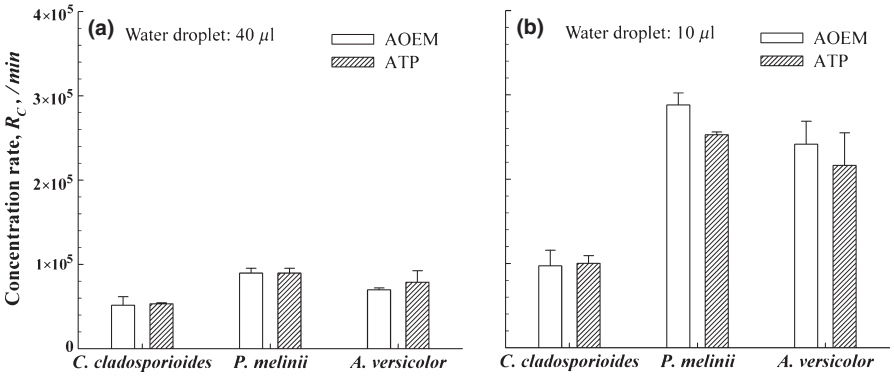

Fig. 6 Comparison of concentration rate of the EPSS when sampling fungal spores (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) based on the 1st collecting water droplet (40 and 10 μl) at 10 l/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage. The error bars represent the standard deviation from three repeats

(approximately 0.9 μm in diameter) and PSL particles (approximately 2 μm in diameter), but still higher than concentration rates of currently available bioaerosol samplers. In addition, these values were observed for a particular reference concentration of airborne spores (approximately 10^5–10^6/m^3). In the ambient environment, spore concentrations can vary widely and in most cases are approximately 10^4/m^3, but concentrations as high as 10^7/m^3 could be encountered (Tsai et al., 2007).

As the concentration of fungal spores in an ambient environment could vary widely, in the next set of experiments, we explored the effect of airborne spore concentration (10^4 to $\sim 10^7$/m^3) on the collection efficiency of the EPSS (Figure 7). Here, the total number of spores collected in a 40-μl droplet is plotted as a function of the reference spore number, $N_{reference}$, determined according to Equation 3. The reference spore number represents the number of spores entering the sampler during a collection period. As the 40-μl droplet yielded higher collection efficiencies compared to the 10-μl droplet, only the 40-μl droplet was used. Because of the limited sensitivity of AOEM at lower

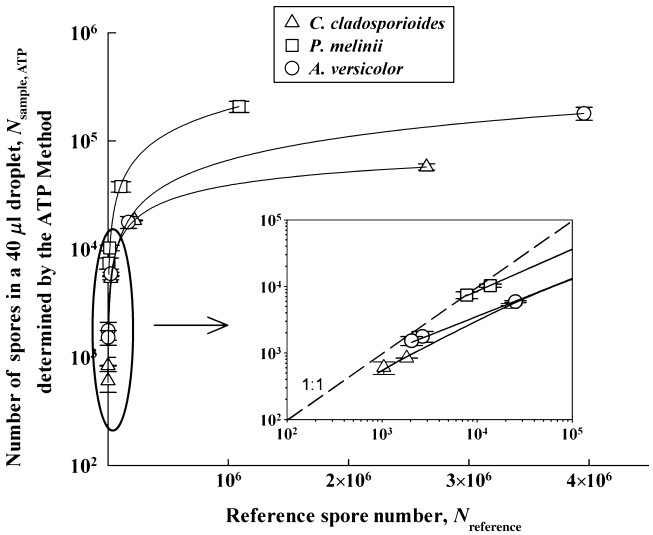

Fig. 7 Total number of spores collected in a 40-μl droplet as a function of the reference spore number, $N_{reference}$, at 10 l/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage with *Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*. The error bars represent the standard deviation from three repeats

concentrations, sample analysis was performed only using the ATP-based method. The reference spore number was determined via APS.

The large figure (Figure 7) shows the entire tested range, while the insert figure shows the results for lower reference spore numbers. It is apparent that for common concentrations of ambient airborne fungi (approximately 10^4–10^5/m^3, $N_{reference} = 10^3$ to $\sim 10^4$), the number of spores recovered in a droplet is closer to the 1:1 line and the sampler collection efficiency and the resulting concentration rate would be substantially higher than those shown in Figures 5 and 6. In fact, for the lowest reference spore numbers tested, the collection efficiency exceeded 60% for all fungal species. As the reference spore number increases, a progressively lower percentage of spores are recovered by the droplet – i.e., the collection efficiency of the EPSS becomes progressively lower. The rate of change, however, seems to depend on the fungal species. For the airborne reference spore number, $N_{reference} = 10^6$ (airborne concentrations = approximately 10^7/m^3), which was the highest tested, the collection efficiencies for *C. cladosporioides* and *A. versicolor* were under 5%, while the collection efficiency for *P. melinii* was approximately 20%. Thus, this sampling technology may not be best suited for sampling of very high airborne spore concentrations. On the other hand, ambient concentrations of fungi of 10^4/l, or 10^7/m^3, are rarely encountered, and the primary application of the EPSS is the detection of low bioaerosol concentrations because of its high concentration rates.

The results presented in Figure 7 could be explained based on the hygroscopicity of the fungal spores. Other physical characteristics of spores, such as size, shape, surface features, density, and electrostatic charge have also possibly played a role. It is generally accepted that fungal spores are hydrophobic (Reponen et al., 2005). Thus, when the collecting water droplet moves over the spores deposited on the collection electrode, majority of the spores attach to the droplet and do not penetrate into it, as shown in Figure 8. More hydrophilic particles, such as bacteria, on the other hand, penetrate into the droplet. At low numbers of deposited spores, the majority of spores can attach to the droplet, which results in high collection efficiency. At high numbers of deposited spores, the surface of the water droplet quickly becomes saturated and cannot accept any more fungal spores. As a result, the collection efficiency of the EPSS substantially decreases for higher spore concentrations. Theoretically, 10^6–5×10^6 fungal spores with a diameter of 2–3 μm could be uniformly packed onto the surface of a 40-μl droplet, while approximately 10^9 spores would fit inside such a droplet. The highest number of spores we were able to collect was in the order of 10^5 to $\sim 10^6$ spores in a 40-μl droplet. Thus, at high reference spore numbers, we were approaching the saturation limit of the droplet surface, i.e., many of the spores deposited on the collection electrode were not picked up by the droplet.

Naturally, not all spores will accumulate only on the droplet surface, but some will penetrate into the droplet. The ratio of spores on the droplet vs. spores inside the droplet is most likely determined by the hydrophobic properties of each individual spore type (Figure 8). As could be inferred from Figure 7, the maximum number of spores that could be accommodated by a droplet is different for each spore type and possibly represents a difference in their hydrophobicity. Based on this reasoning, the *C. cladosporioides* spores seem to be most hydrophobic, while the *P. melinii* spores seem to be least hydrophobic of the three tested species. The data presented in Figure 5 also show that the highest collection efficiency was observed for *P. melinii* spores and the lowest for *C. cladosporioides* spores.

The tests described previously were performed with a 10-min sampling time. For many sampling projects, longer sampling times are needed and thus additional tests were performed for the 30- and 60-min sampling times. Given the data presented in Figure 7, i.e., decrease in the collection efficiency at higher spore loads, the airborne spore concentration was adjusted so that the total number of spores entering the EPSS would be approximately the same for all three

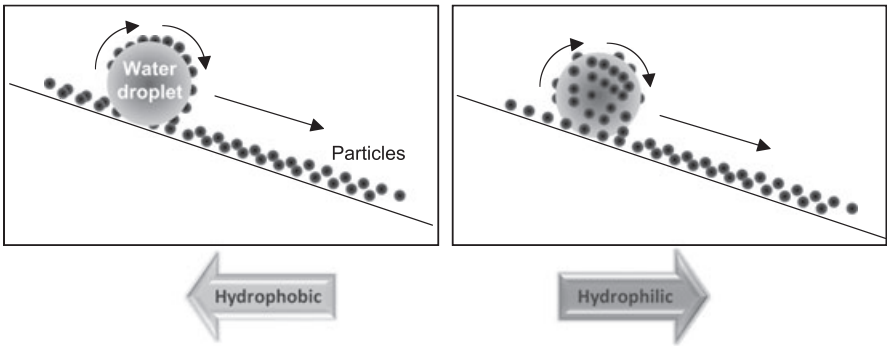

Fig. 8 Schematic illustration of the removal of particles deposited on superhydrophobic surface by a water droplet depending on the hygroscopicity of particles

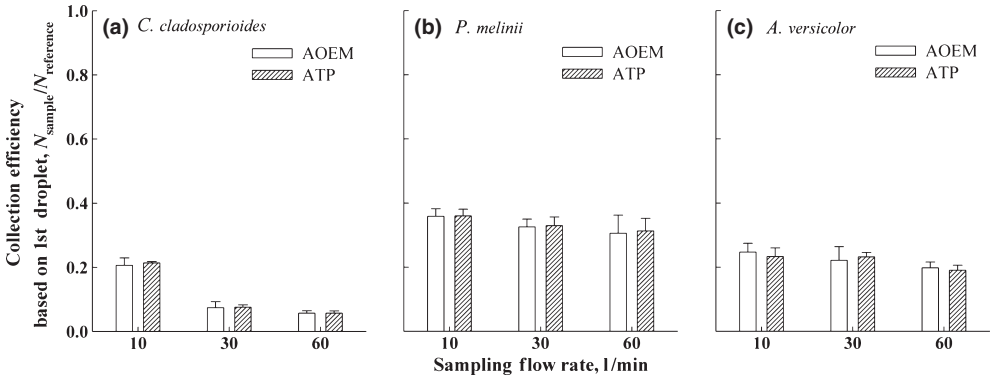

Fig. 9 Collection efficiency of the EPSS when sampling fungal spores (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) based on the 1st collecting water droplet (40 μl) as a function of sampling time (10, 30, and 60 min) at 10 l/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage. The error bars represent the standard deviation from three repeats

sampling times. The collection efficiency of the EPSS tested at 10 l/min with the 40-μl droplet for a 10-, 30-, and 60-min sampling time is presented in Figure 9. The collected spores were quantified by both the AOEM and the ATP-based method. When the sampling time was increased from 10 to 60 min, the EPSS collection efficiency for *C. cladosporioides* analyzed by the AOEM decreased from 21 ± 4% (21 ± 0.4% by ATP) to 6 ± 0.8% (6 ± 0.7% by ATP). The decrease for the other two fungal species was much less pronounced: for *P. melinii*, from 36 ± 2.4% (36 ± 2.4% by ATP) to 31 ± 5.7% (31 ± 3.9% by ATP); for *A. versicolor,* from 25 ± 2.8% (23 ± 2.7% by ATP) to 20 ± 1.8% (19 ± 1.6% by ATP). The decrease in the collection efficiency with a longer sampling time was also observed in our earlier study with bacteria (Han et al., 2010). One possible explanation of the decrease could be less efficient removal of the deposited particles by water droplet because of the increase in the adhesive forces between the particles and the collection surface with prolonged contact time (Hinds, 1999). The increase in the adhesion force seems to depend on the particle type and in our case seems to have been most pronounced for *C. cladosporioides*.

Another possible contributing factor is the loss of electrical charge with time and thus reentry of the collected spores into the air stream. However, even if the spores did lose most of their charge and the contribution of electrostatic force to the overall adhesive force became minimal, the adhesive force for particles < 10 μm would still be much higher than a removing force of an air current of 10 m/s (Hinds, 1999), which is stronger than the air current used in our experiments by more than an order of magnitude. Accordingly, the fraction of pores observed on the after-filter did not increase with the longer sampling time.

Even though the average collection efficiency for three fungi after a 60-min sampling time was approximately 20%, note that all particles removed from the EPSS are concentrated in only one droplet. Therefore, the entire sample could be analyzed thus increasing the ability to detect particles of interest. As could be observed from Figure 9, the average collection efficiency determined using the two methods (AOEM vs. ATP) was in a very good agreement: the difference was not statistically significant ($P > 0.05$) for either the different fungal species nor for different sampling times.

Conclusions

This study showed that the novel bioaerosol sampler with a superhydrophobic collection surface is able to concentrate airborne fungi (*C. cladosporioides*, *P. melinii*, and *A. versicolor*) in small amounts of liquid (10 or 40 μl). When sampling with 10-μl droplets, concentration rates in the range of 1×10^5–3×10^5/min were achieved even though the collection efficiency was $< 50\%$. Such concentration rates are higher than those of the most currently available bioaerosol samplers, thus improving the detection of low concentrations of airborne fungi in various environments, including indoors. In addition, it was demonstrated that the collection efficiency of the EPSS substantially increases at lower spore loadings and is $> 60\%$ for airborne spore concentrations usually encountered in the environment, i.e., 10^4 to $\sim 10^5/m^3$. Based on this study with fungi and an earlier study with bacteria and PSL particles, it seems that the performance of this sampling technology depends on particle nature and interaction between the collected particles and the superhydrophobic collection surface. Among these particles, fungi represent the worst case scenario. As airborne fungal spores might constitute only 1–4% of particles outdoors (Battarbee et al., 1997), it is likely that $> 10^6$ to $\sim 10^7$ particles could be removed by one 40-μl droplet during field sampling. The interaction of different particles with the collection surface will be investigated in future studies in more detail.

In addition, we successfully developed and applied an ATP-based bioluminescence method to quantify collected fungal spores. Spore concentrations determined by the microscopy and the ATP-based method were in very good agreement and were not statistically different. It should be noted that that these results were achieved with pure fungal cultures. In a field sampling situation, where different genera of bacteria, fungi, and their fragments are encountered in variable stages of dormancy/germination, the ATP signal could not be correlated with concentration of a particular bioaerosol species. In this case, the ATP signal may serve as an indicator of the overall viable bioaerosol burden. On the other hand, the ATP method could serve as a fast and quantitative alternative to traditional microscopy in laboratory studies with specific fungal species.

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