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Investigation of Cut-Off Sizes and Collection Efficiencies of Portable Microbial Samplers

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This research investigated the physical collection efficiencies and cut-off sizes of SMA MicroPortable, BioCulture, Microflow, Microbiological Air Sampler (MAS-100), Millipore Air Tester (MAT), SAS Super 180, and RCS High Flow portable microbial samplers when collecting Polystyrene Latex particles ranging from 0.5 to 9.8 μm in aerodynamic size.

Traditional collection efficiency measurements often directly compare particle concentrations upstream and downstream of the sampler without considering the particle losses. Here, we developed a new approach which tests collection efficiencies of the sampler with and without agar collection plate loaded. This method thus allows estimating the effective collection efficiency, i.e., the fraction of incoming particles deposited onto the agar collection medium only.

The experimental cut-off sizes, or d_{50}, of the investigated samplers ranged from 1.2 μm for the RCS High Flow, 1.7 μm for the MAS-100, 2.1 μm for SAS Super 180, to 2.3 μm for MAT; for other three samplers they were close to or above 5 μm. In most cases the theoretical d_{50} was lower than the experimental value, which was likely due to the dissipation of impactor jets and the influence of cross-flow in the multi-nozzle impactors. For most samplers, we observed a notable difference between the collection efficiency obtained by the traditional measurement method and the effective collection efficiency. In general, all samplers collected 10% or less of 0.5 μm particles onto the agar medium.

This study indicates that the use of most of the tested bioaerosol samplers may result in a substantial underestimation of bacterial concentrations, especially of single bacterial cells with diameter 0.5–1.0 μm. On the other hand, most of the investigated samplers would be more efficient when collecting larger fungal spores.

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INTRODUCTION

The inhalation of airborne biological agents, ranging from submicron allergens to larger bacteria, fungi, and pollen may result in adverse health effects, such as respiratory symptoms and lung function impairments (Douwes et al. 2003). The onset of the Sick Building Syndrome is thought to be at least partially due to the exposure to the airborne biological agents (Walinder et al. 2001; Cooley et al. 1998; Teeuw et al. 1994). Excessive mold growth in damp building has been associated with irritation, allergy, and infections among building occupants (Fung and Hughson 2003; Koskinen et al. 1999; Dales et al. 1991). Adverse health effects due to bioaerosol exposure have been observed in waste recycling (van Tongeren et al. 1997; Douwes et al. 2000; Bünger et al. 2000), food processing, and detergent industries (Sandiford et al. 1994; Schweigert et al. 2000). In addition, the increased threat of bioterrorism poses a significant health concern both for government and private sectors. Adequate address of various health challenges presented by bioaerosols requires qualitative and quantitative assessment of the bio-burden in indoor and outdoor environments and application of advanced bioaerosol sampling methods and tools.

One of the key limitations in bioaerosol sampling is sampler's portability. Commonly used samplers, such as Andersen impactor (Thermo Andersen Corp., Franklin, MA) and AGI-30 impinger (Ace Glass Inc., Vineland, NJ) often require external power source and can not be easily applied in remote locations (National Research Council 2003). Some Andersen-type impactors, such as BioStage (SKC Inc., Eighty Four, PA), however, have already been adapted for use with portable pumps. In addition, detection of low microorganism concentrations often requires sampling flow rates larger than provided by classical samplers. Therefore, microbial samplers capable of drawing air at flow rates of 100 L/min and higher while still being portable are currently getting popular for monitoring biological agents. A number of such samplers have already been used in field investigations or discussed in literature (Berardi et al. 1991; Der et al. 2005; Burge et al. 2000; Li and Lin 1999; Lee and Chang 2000; Nesa et al. 2001; Dacarro et al. 2005; Martinez et al. 2004; Powitz et al. 2002; Távora et al. 2003). In addition, every year new or redesigned models are introduced into the market.

When selecting a particular sampler for collecting microbial agents of concern, it is important to know its cut-off size, or d_{50}. The last stage of commonly used Andersen impactor has a cut-off size of 0.65 μm, while an AGI-30 impinger has a cut-off size of 0.3 μm (Research Triangle Institute 2004). Such cut-off sizes allow these samplers to collect most of the airborne single bacterial cells and fungal spores. The information about cut-off sizes and collection performance, however, is not readily available for most of the portable microbial samplers, especially for the newer sampler models. Therefore, the main goal of this study was to investigate the collection efficiencies of several portable microbial samplers, to determine their cut-off sizes and to compare these values with theoretical estimates. In addition, we determined not only the fraction of airborne particles deposited on the agar collection medium (Effective Collection Efficiency), but also the fraction of airborne particles collected by the air mover (Air Mover Collection Efficiency), which is often not reported. Also, for some samplers, the collection efficiency was investigated as a function of sampling flow rate and the amount of collection medium (agar).

METHODS AND MATERIAL

Test Samplers

The samplers tested in this research included MAS-100 (EMD Chemicals, Inc., Gibbstown, NJ), Microflow (Aquaria srl, Lacchiarella, Italy), BioCulture (A.P. BUCK Inc., Orlando, FL), SMA MicroPortable (Veltek Associates, Inc, Phoenixville, PA), SAS Super 180 (Bioscience International, Inc., Rockville, MD), Millipore Air Tester (Millipore Corp., Billerica, MA), and RCS High Flow (Biotest Diagnostics Corp., Denville, NJ). All these samplers are impactors collecting particles onto a single agar plate. The only exception is the RCS High Flow, which collects particles onto proprietary agar strips supplied by the manufacturer. The physical characteristics of the samplers, such as sampling flow rate, number of nozzles, nozzle diameter, jet-to-plate distances tested, amount of agar used, throat length, and S/W ratio (jet-to-plate distance/nozzle diameter), and Reynolds numbers are listed in Table 1.

The dominant parameter describing the collection efficiency of an impactor is the Stokes number. The recommended square root value of Stokes number corresponding to d_{50} is 0.49 for circular jet (Hinds 1999). Theoretical estimates of the cut-off size, d_{50}, of the portable microbial samplers evaluated in this study were determined using the following equation (Marple et al. 1993):

$$d_{50} = \sqrt{\frac{9\eta W}{\rho_p U_0 C_c}}\sqrt{\mathrm{Stk}_{50}}, \qquad [1]$$

where η is the air viscosity, W is the diameter of the impactor nozzle, $\sqrt{\mathrm{Stk}_{50}}$ is the square root of Stokes number for the collection efficiency of 50%, ρ_p is the particle density, U_0 is the jet velocity through the impactor nozzle, and C_c is the Cunningham correction factor. In addition, S/W ratio, jet throat length and Reynolds number Re might also play a role in shaping the characteristic collection efficiency curve of an impactor (Marple and Willeke 1976; Marple et al. 1993; Hinds 1999). For certain samplers, the theoretical d_{50} was calculated for different sampling flow rates, or different jet velocities through the impactor nozzle, U_0.

Different from other impactors, the primary collection mechanism in RCS High Flow sampler is based on the centrifugal force. By equating centrifugal force to drag force as suggested by Mitchell (1995), we can determine terminal velocity V_T towards agar strip for particles of certain size:

$$V_T = \frac{C_C d^2 \rho_p V_i^2}{18\eta R}, \qquad [2]$$

where V_i is the inlet velocity into centrifugal drum, and R is the rotation radius. Since air density is much lower than particle density, it is omitted here for simplicity. Once the V_T is known, we can adapt the cyclone collection theory suggested by Schnelle and Brown (2002). Assuming a uniform particle distribution in the distance between the inner radius of the drum and agar strip ($D = 3$ mm), particles have to travel half of that distance ($0.5D$) during their residence time in the vortex for 50% of them to be collected. Residence time in the vortex depends on the rotation radius R and the number of effective turns, N_e. Since the air stream entering the centrifugal drum has a width $w \approx 0.7$ cm and the overall width of RCS High Flow's centrifugal drum is $W \approx 2.1$ cm, we estimate that $N_e \approx 3$. The W here is the same as the width of the agar strip. By taking into account the needed travel time towards the agar strip and the terminal particle velocity we can estimate the d_{50} of RCS High Flow sampler as:

$$d_{50} = \sqrt{\frac{9\eta D}{2\pi N_e C_c V_i \rho_p}} \qquad [3]$$

where V_i is the inlet velocity into the centrifugal drum. For the given w and D, the V_i is 7.9 m/s based on the flow rate of 10 L/min (assuming the total sampling flow rate of 100 L/min evenly divides among 10 impeller partitions).

Test Particles

Polystyrene Latex (PSL) particles (Bangs Laboratories, Inc., Fishers, IN) were used to evaluate the physical collection efficiencies and cut-off sizes of the tested portable microbial samplers. The test particles had mean aerodynamic sizes of 0.49, 0.97, 1.95, 2.95, 3.62, and 5.22 μm. Some samplers have also been tested with 9.8 μm PSL particles. The selected size range includes majority of the airborne bacteria and fungi.

TABLE 1
Physical characteristics of tested portable microbial samplers

Sampler	Sampling flow rate, L/Min	# of impaction nozzles	Nozzle diameter, W, mm	Throat length, mm	Cross-flow parameter	Amount of Agar used, mL	Jet-to-plate distance, S, mm	S/W ratio	Reynolds number	Theoretical d_{50}, μm	Experimental d_{50}, μm
SMA	28.3	12	6.3	9.3	0.3	25	5	0.8	528	13.7	>10
	141.5	12	6.3	9.3		25	5	0.8	2638	6.0	4.8
BioCulture	120	380	1.25 (outer diameter) 2.3 (inner diameter)	2.5	3.1	30	1.7	0.75	193 (for inner diameter)	8.13 (for inner diameter)	7
MAS-100	100	400	0.7	3.1	1.06	50	2.8	4	503	1.47	1.7
	100	400	0.7	3.1		30	6.4	9	503	1.47	2.5
Microflow	30	378	1.1	3.1	3.4	25	1.89	0.84	45–180	17.5	>10
	120	378	(outer diameter) 2.5 (inner diameter)	3.1		25	1.89	0.84	(for inner diameter)	8.7 (for inner diameter)	8.8
SAS Super 180	180	401	0.8	2.4	1.06	40	2.16	2.7	791	1.3	2.1
	180	401	0.8	2.4		25	4.7	6	791	1.3	3.0
Millipore Air Tester	140	1000	0.46	0.8	1.7	Plate supplied by	5.84	12.7	423	1	2.3
	180	1000	0.46	0.8		manufacturer	5.84	12.7	544	0.9	2.5
RCS High Flow	100	N/A	N/A	N/A	N/A	Agar strip supplied by manufacturer	N/A	N/A	N/A	~1.7	1.2

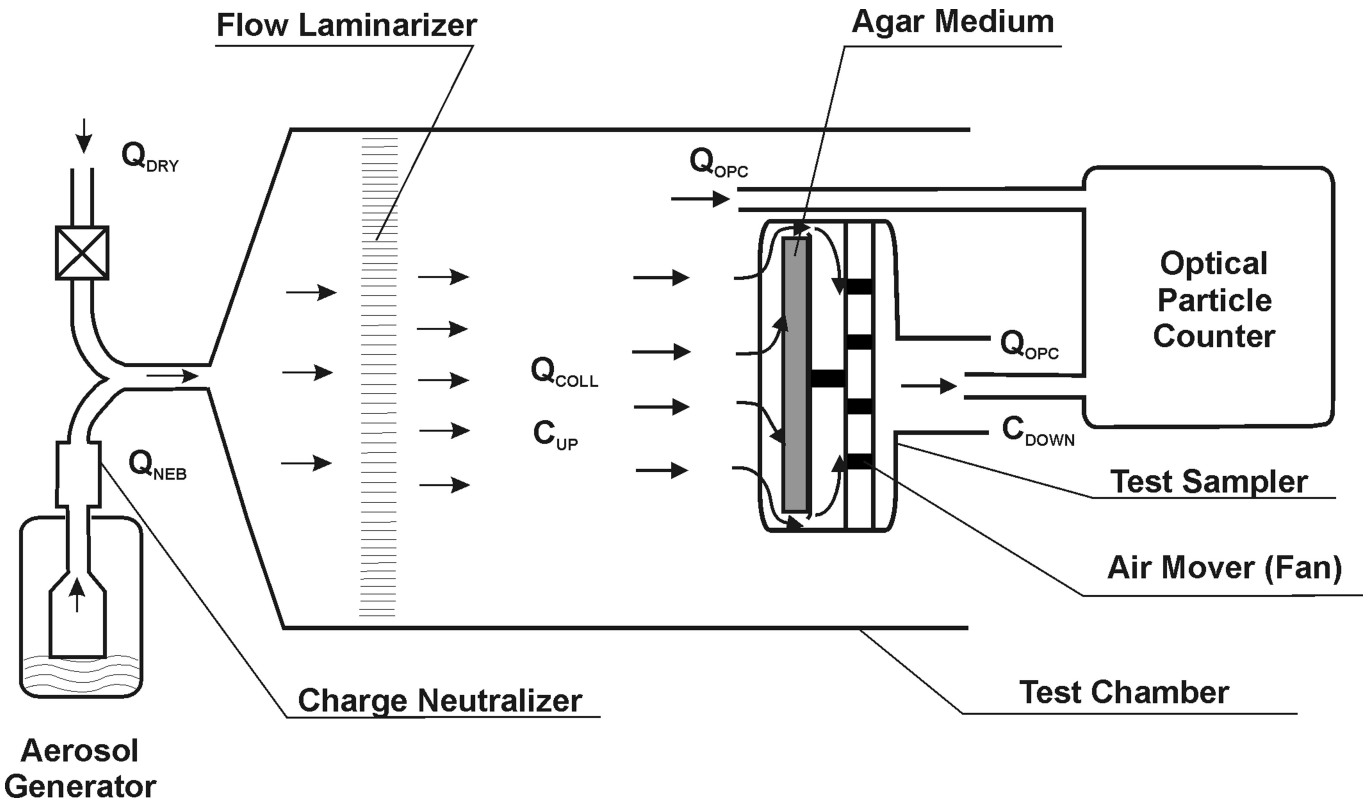

FIG. 1. A schematic representation of the experimental setup.

Experimental Setup

The experimental setup used in this research is shown in Figure 1. The Collison six-jet nebulizer was used to aerosolize PSL particles suspended in freshly purified water (Mili-Q system, Millipore, Billerica, MA). The aerosolization flow rate was varied between 3 and 10 L/min to achieve the desired particle concentrations. The concentration of test particles smaller than 3 μm was on the order of 5,000 particles/L, while concentration of particles larger than 3 μm was on the order of 1,000–3,000 particles/L. The aerosolized particles passed through the Po-210 charge neutralizer and were then carried into the test chamber by the dry air flow $Q_{\mathrm{DRY}} = 400$ L/min. This flow rate resulted in the air flow velocity of 22 cm/s inside the testing chamber thus simulating an indoor environment where air is usually moving at velocities less than 30 cm/s (Berry and Froude 1989; Baldwin and Maynard 1998). Such criteria were selected because in most applications the portable microbial samplers are used in occupational and residential indoor environments. A microbial sampler under investigation was placed in the test chamber and the air sampling nozzles connected to an Optical Particle Counter, OPC (model 1.108, Grimm Technologies Inc., Douglasville, GA) were placed at the inlet and outlet of the sampler to isokinetically measure particle concentrations upstream and downstream of the sampler. The isokinetic sampling is achieved when the mean air flow velocity through the sampling nozzle is equal to the air flow velocity of the environment being tested: for the upstream measurements it was matched with the air flow velocity in the chamber; while for the downstream measurements it was matched with velocity of air leaving the sampler. To account for possible downstream air flow disruption by the fan's centrifugal forces, the velocity of air leaving the sampler was calculated as the average air velocity across the outlet. The OPC used in this study operated at a flow rate $Q_{\mathrm{OPC}} = 1.2$ L/min.

Determination of the Collection Efficiency

The comparison of particle number concentration entering and leaving a sampler for determining its overall collection efficiency is a well-established methodology and has been used in previous studies (Agranovski et al. 2002; Phan and McFarland 2004). However, the direct comparison of the particle concentrations upstream and downstream of the investigated sampler would overestimate the percentage of particles actually collected onto agar media, i.e., the effective collection efficiency, because some particles not collected by agar might be collected by an air mover as shown in Figure 1. To address this concern, we treated a sampler as a particle collector with two collection stages: collection by the agar media and subsequent collection by the air mover, and developed a test procedure that allows differentiating between the collection efficiency by agar media and the collection efficiency by air mover.

In this procedure, the collection efficiency of the sampler was determined both with and without agar collection plate loaded. When the agar collection plate is loaded into the sampler, the determined collection efficiency of the sampler is described as the Overall Collection Efficiency, $E_{agar+fan}$, i.e., that of agar medium and air mover together. When the agar collection plate is not loaded into the sampler, the determined collection efficiency is described as the Air Mover Collection Efficiency, E_{fan}. The particle collection efficiency of the sampler was measured with and without agar collection plate loaded using the following formula:

$$E_{COLL} = \left(1 - \frac{C_{DOWN}}{C_{UP}}\right) \times 100\%, \quad [4]$$

where E_{COLL} is the collection efficiency of the sampler, i.e., $E_{agar+fan}$ or E_{fan}, with and without agar collection plate loaded, respectively; C_{UP} and C_{DOWN} are particle concentrations entering and leaving the sampler.

Since the air sampler is treated as a two-stage collection system, then its Overall Collection Efficiency could be described as

$$E_{agar+fan} = (1 - [(1 - E_{agar})(1 - E_{fan})]) \times 100\%, \quad [5]$$

where the E_{agar} is the percentage of the airborne particles removed onto the agar collection plate, or Effective Collection Efficiency. The sampler's Effective Collection Efficiency, E_{agar}, can then be calculated using the following equation:

$$E_{agar} = \left(1 - \frac{1 - E_{agar+fan}}{1 - E_{fan}}\right) \times 100\%. \quad [6]$$

The E_{agar} indicates the fraction of particles collected on the agar collection plate and not elsewhere in the sampler.

Removal of the agar collection plate to measure the E_{fan} might have affected the resistance to the air flow through the sampler and thus the total air flow rate through the sampler. Therefore, we measured the samplers' inlet air velocities at several locations across the sampling inlet using Traceable Hot Wire Anemometer/Thermometer (Control Company, Friendswood, TX) and calculated corresponding sampling flow rates with and without agar collection plate loaded and found that the flow rate difference was within 5%.

The only exception to the procedure above was the RCS High Flow sampler which is a centrifugal impactor collecting particles on agar strips and does not have an air mover positioned behind the agar collection strip. Therefore the RCS High Flow was tested only with the agar strip loaded. Since this sampler does not have the "second collection stage," we assumed that its Overall Collection Efficiency, $E_{agar+fan}$, approximates its Effective Collection Efficiency, E_{agar}.

During the testing, each repeat with each microbial sampler was performed with a new agar collection plate. The samplers were tested using trypticase soy agar (Becton, Dickson and Company, Sparks, MD), which has 1.5% solid agar. Each measurement upstream or downstream of the sampler lasted 1–2 minutes to minimize the desiccation of the agar collection medium. The standard deviations of $E_{agar+fan}$ and E_{fan} were calculated from at least three repeats. Since the E_{agar} is based on calculation, we used a partial differential equation method described by Ku (1966) to propagate the errors, which is shown in all figures involving the Effective Collection Efficiency.

RESULTS AND DISCUSSION

This study has shown that the physical collection efficiencies ($E_{agar+fan}$, E_{fan}, and E_{agar}) and the cut-off sizes, d_{50}, of the tested portable samplers varied substantially depending on the sampler model. The experimental collection efficiency was also found to depend on the sampling flow rate and the amount of collection medium, which determined the jet-to-plate distance. For most of the investigated multi-nozzle samplers, there was a difference between the theoretical d_{50} estimates, which are determined for ideal air flow conditions through a single nozzle, and the experimental cut-off sizes.

Experimental tests with SMA MicroPortable sampler indicated that the d_{50} is approximately 4.8 μm for sampling flow rate of 141.5 L/min, and higher than 10 μm for sampling flow rate of 28.3 L/min as shown in Figure 2. The theoretical estimates of the cut-off size of the sampler using Equation (1) are 6 and 13.7 μm for its sampling flow rates of 141.5 and 28.3L/min, respectively. 50% of 5 μm particles were collected on agar at 141.5 L/min, while only about 18% of such particles are collected at 28.3 L/min. The collection efficiencies for particles smaller than 3 μm were rather low, which would lead to underestimations of airborne bacterial concentrations since size of most single bacteria range from 0.5 to 3.0 μm. The sampler might be more effective when collecting bacterial and fungal agglomerates. The S/W ratio for SMA MicroPortable, i.e., the ratio of the jet-to-plate distance over the diameter of the impactor nozzle, is 0.8, which is less than the recommended value of 1.0 or above for round impactors (Hinds 1999).

Tests with BioCulture microbial sampler at 120 L/min showed that the d_{50} was approximately 7 μm as shown in Figure 3. Theoretical estimate of the BioCulture's cut-off size is about 8.13 μm at the sampling flow rate of 120 L/min, if inner nozzle diameter is used for calculations and 3.3 μm if outer nozzle diameter is used. When tested with 5.2 μm particles at sampling flow rates lower than 120 L/min, the E_{agar} decreased, and at 30 L/min it was virtually zero. This result indicates that very few particles of 5.2 μm and smaller would be collected at this sampling flow rate. The size of 5.2 μm represents the upper size range of commonly encountered bacteria and fungi.

Since no specific amount of agar for MAS-100 was recommended by the manufacturer, we tested the sampler with two amounts of agar: 30 and 50 mL as indicated in Figure 4. The theoretical estimate of the cut-off size, d_{50}, for MAS-100 is about 1.5 μm. When 30 mL agar was used, the S/W ratio was 9 which is beyond the recommended range of 1–5 (Hinds 1999). The

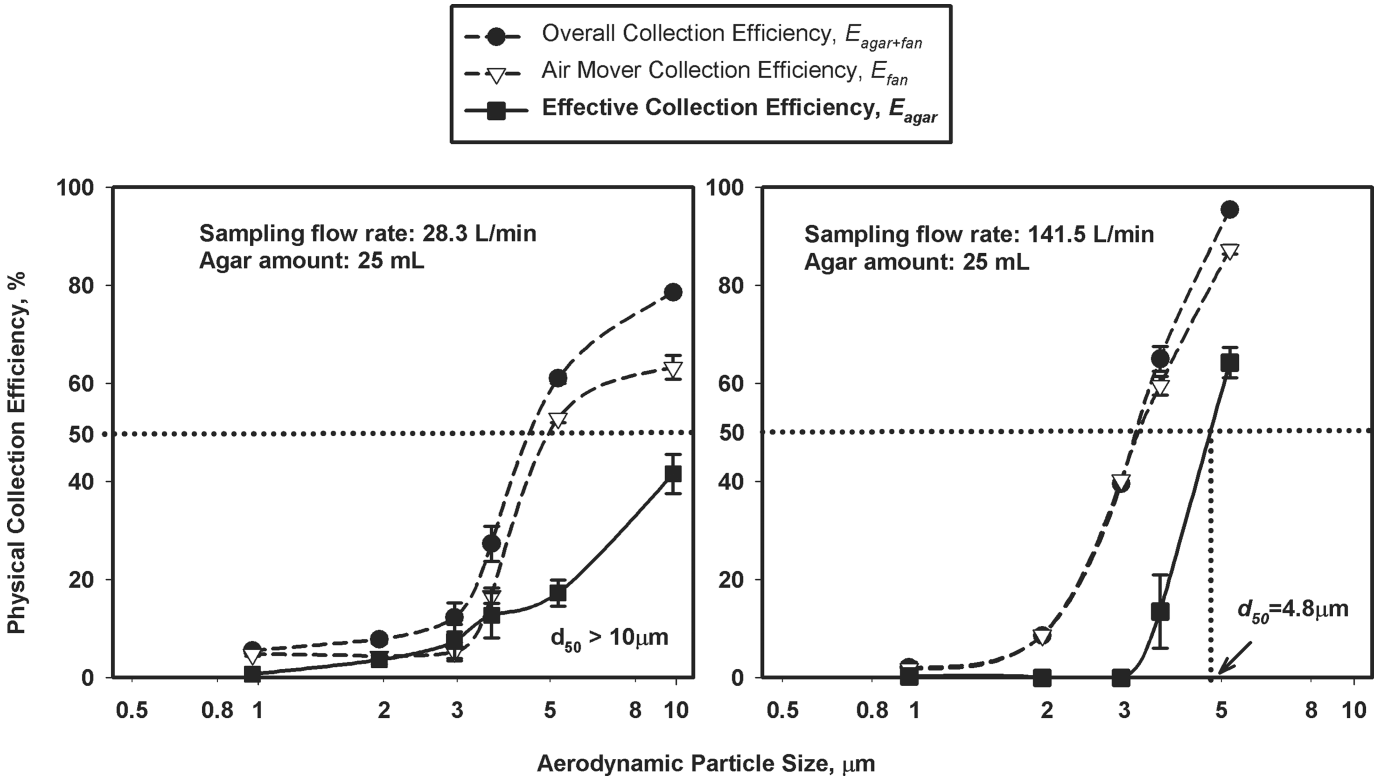

FIG. 2. Physical collection efficiencies of SMA MicroPortable microbial sampler at different sampling conditions. Data represent averages of three repeats and error bars stand for 1 standard deviation.

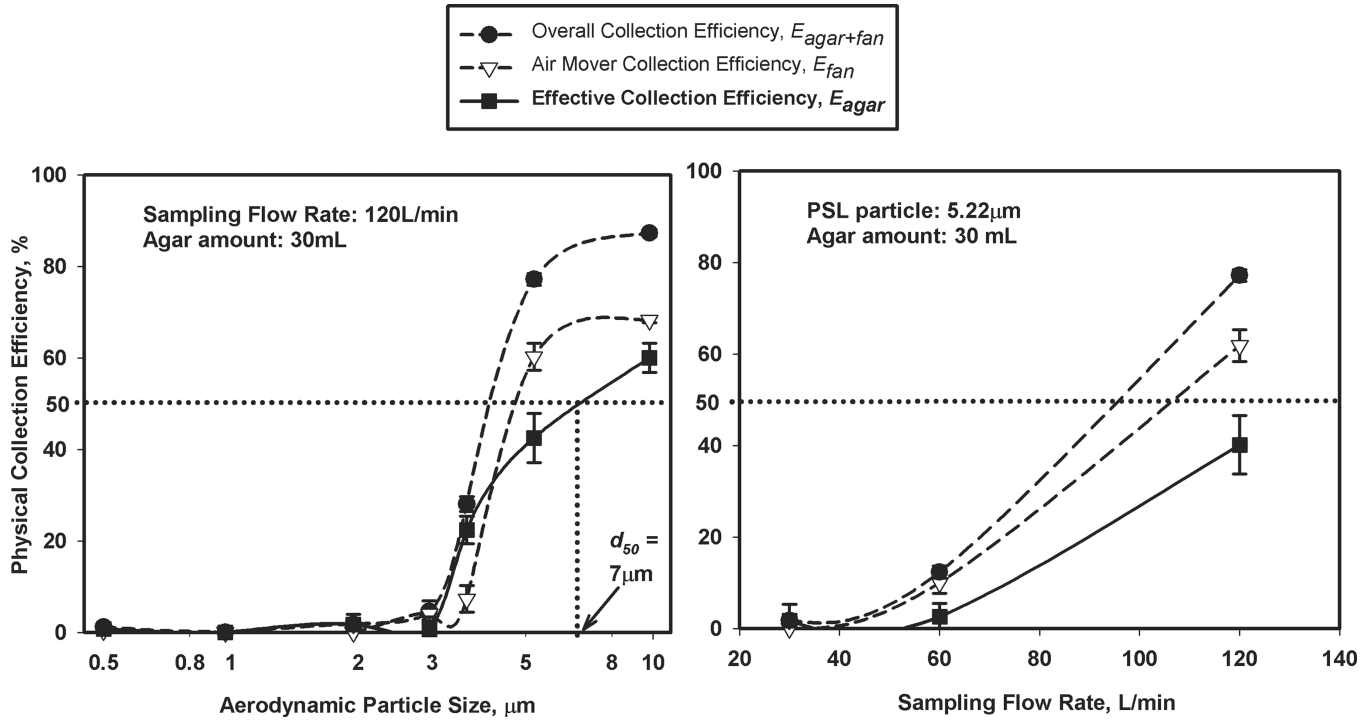

FIG. 3. Physical collection efficiencies of BioCulture microbial sampler at different sampling conditions. The data represent averages from three repeats and error bars stand for 1 standard deviation.

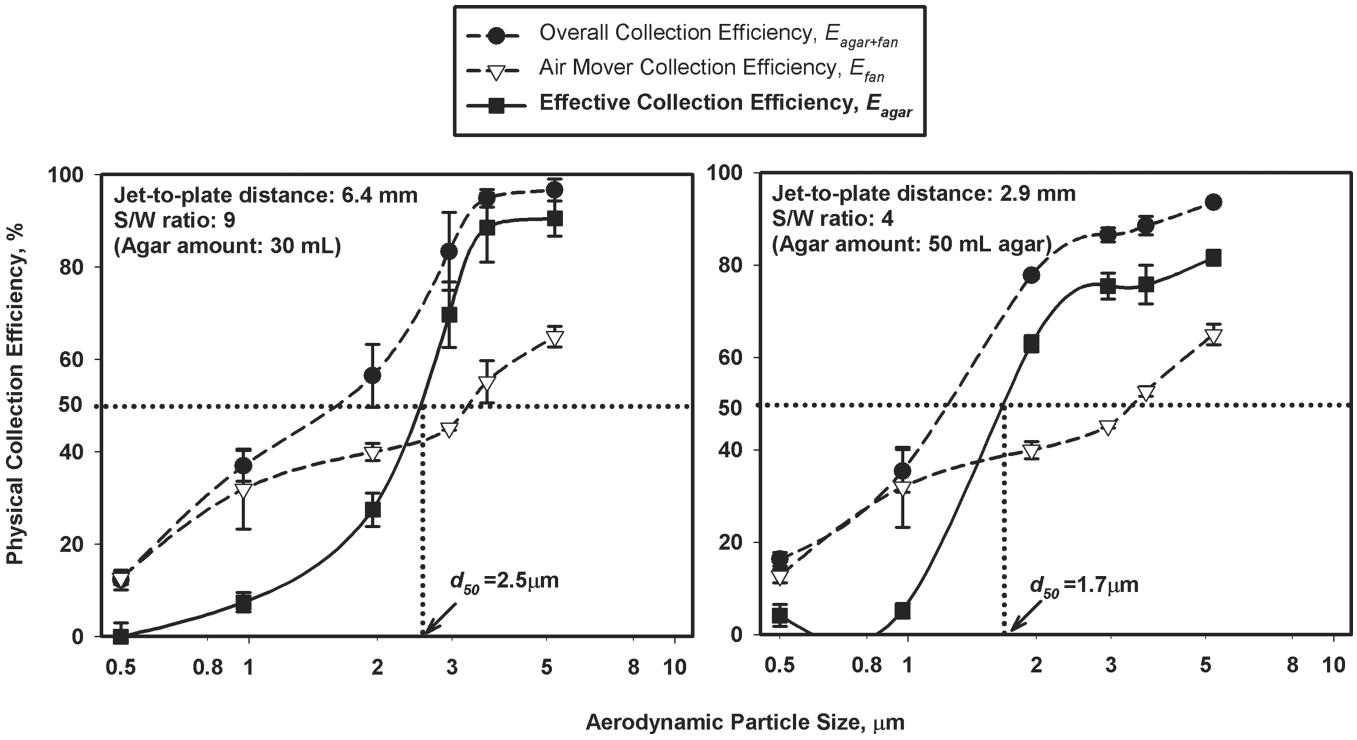

FIG. 4. Physical collection efficiencies of MAS-100 microbial sampler at different jet-to-plate distances. The data represent averages from three repeats and error bars stand for 1 standard deviation.

experimental d_{50} in this case was 2.5 μm (Figure 4), which is substantially higher than the theoretical estimate. The experimental d_{50} moved much closer to the theoretical estimate when the amount of agar per Petri dish was increased to 50 mL. In this case, the d_{50} was 1.7 μm and the jet-to-plate distance was 2.8 mm with a S/W ratio of 4 which is within the recommended range of 1.0–5.0 (Hinds 1999).

For MAS-100 impactor, the jets apparently dissipate with increasing jet-to-plate distance and its E_{agar} decreases. The Effective Collection Efficiency, E_{agar}, of MAS-100 is above 60% for particles of 2 μm when the jet-to-plate distance is 2.8 mm (50 mL of agar), but it decreases to 30% for the same particles when the jet-to-plate distance is 6.4 mm (30 mL of agar) as shown in Figure 4. When sampling particles of 3 μm and larger, the E_{agar} is somewhat lower for S/W = 4 compared with S/W = 9. We believe that for S/W = 4, the particles have sufficient inertia to overcome dissipation of the impaction jets, but at the same time experience increased bounce which results in lower E_{agar}.

The Microflow sampler's performance when operating at 120 L/min isshown in Figure 5. The sampler's experimental d_{50} was 8.8 μm, which agreed well with the theoretical estimate of 8.7 μm. The jet nozzles of Microflow sampler have a shape of expanding cone with outer diameter of 1.1 mm and the inner diameter of 2.5 mm. When calculating the theoretical d_{50} we used the inner nozzle diameter (side of the nozzle facing the agar) of 2.5 mm. Since this sampler has adjustable flow rates between 30 and 120 L/min, we also tested it at a 30 L/min flow rate. In this case, the sampler's E_{agar} was found to be 8% and 20% for 5.22 and 9.8 μm particles, respectively (data not shown), while its theoretical d_{50} estimate was 17.5 μm. Use of 25 mL agar per Petri dish (as recommended by the manufacturer) resulted in the jet-to-plate distance of 1.9 mm with S/W ratio of 0.84, which is less than the recommended value of 1.0. Overall, at the investigated lowest/highest sampling flow rates of 30 and 120 L/min this sampler seems to collect very few particles smaller than 3 μm. Such performance would lead to undersampling of most environmental bacteria and fungi.

The data with SAS Super 180 are shown in Figure 6. When the sampler was tested with 25 mL of agar per Petri dish (as recommended by the manufacturer), its jet-to-plate distance was approximately 4.7 mm with the resulting S/W ratio of 6, which is higher than recommended range of 1.0–5.0 (Hinds 1999). The experimental d_{50} based on E_{agar} was 3.0 μm, a much higher value than the theoretical estimate of 1.3 μm. In the next set of experiments we increased the amount of agar to 40 mL which resulted in the jet-to-plate distance of 2.16 mm with S/W ratio of 2.7. The experimental d_{50} in this case decreased to 2.1 μm and the collection efficiency curve became steeper as shown in Figure 6. Overall, the effective collection efficiency of this sampler for the smaller jet-to-plate distance was above 80% for the particles 3 μm and larger. For the larger jet-to-plate distance, the effective collection efficiency is less than 80% even for particles of 5.2 μm. We believe that dissipation of

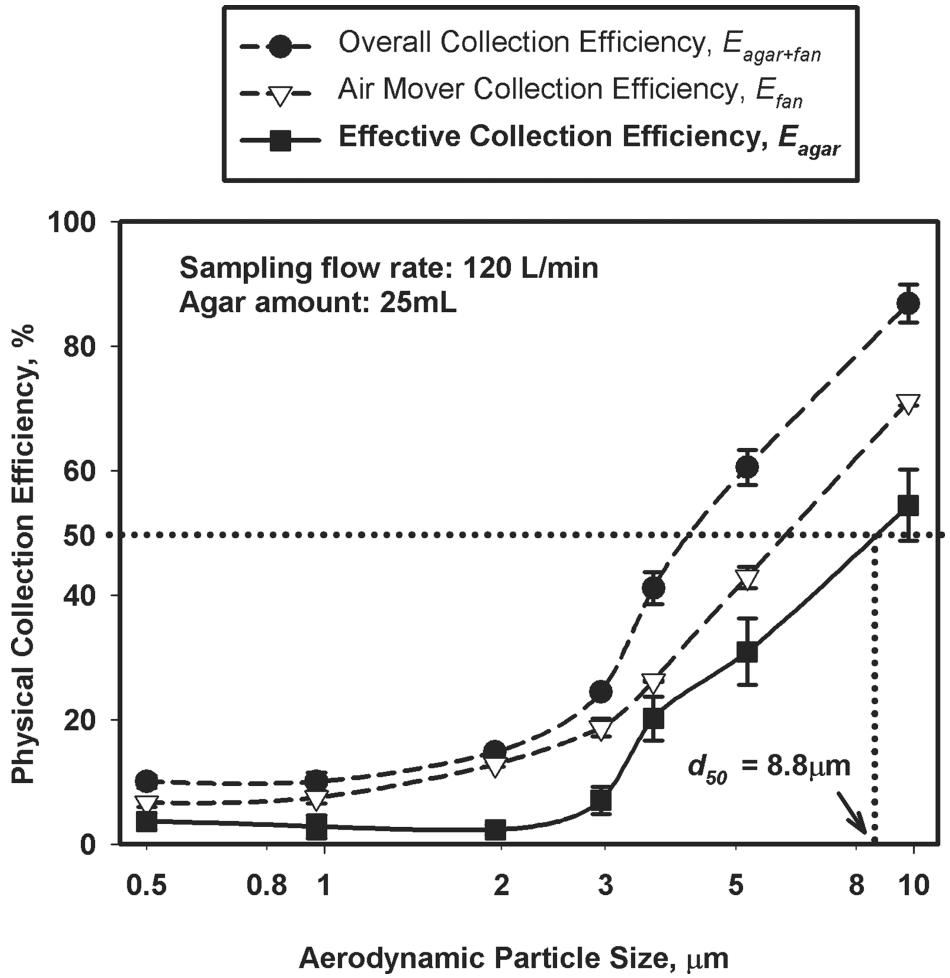

FIG. 5. Physical collection efficiencies of Microflow microbial sampler. Data represent averages of three repeats and error bars stand for 1 standard deviation.

impaction jets at higher jet-to-plate distances causes the decrease in sampler's collection efficiency when 25 mL of agar is used.

The experimental data with the Millipore Air Tester are shown in Figure 7. This microbial impactor has two different sampling flow rates used in sequence. For the first 500 L of air sampled, the sampling flow rate is 140 L/min and then the sampling flow rate automatically switches to 180 L/min. The entire sampling cycle lasts about 6 minutes. This sampler uses manufacturer-prepared agar plates which result in the jet-to-plate distance of 5.84 mm with S/W ratio of 12.7, a much higher value than the recommended range (Hinds 1999). Since the sampler uses two sequential sampling flow rates, we investigated both of them in one complete sampling cycle. Theoretical estimates of the cut-off size, d_{50}, of the sampler are 1.0 μm for 140 L/min sampling flow rate and 0.9 μm for 180 L/min sampling flow rate. According to the experimental data presented in Figure 7, sampler's d_{50} was 2.3 μm and 2.5 μm for sampling flow rates of 140 L/min and 180 L/min, respectively. Different from other samplers, the Millipore Air Tester's collection efficiency seems to have decreased when the sampling flow rate was increased to 180 L/min. This decrease is likely due to the agar desiccation during the sampler's operation at 140 L/min when the first 500 L of air were sampled. The agar desiccation resulted in an increased jet-to-plate distance and hardened agar, which has likely led to an increased particle bounce. As shown in Figure 7, particles of 1 μm in size were collected with efficiencies of approximately 5 % at both sampling flow rates. For PSL particles of 3 μm and larger, the E_{agar} was above 60% when the sampler operated at 140 L/min.

The data with RCS High Flow sampler are shown in Figure 8. The sampler was experimentally observed collecting 1 μm particles with efficiency of 40% and 0.5 μm particles with efficiency of 10%. Unlike other samplers, RCS High Flow does not have an air mover positioned behind the collection surface. Therefore, its E_{agar} was determined based on the Overall Collection Efficiency, $E_{agar+fan}$. Sampler's experimental d_{50} was 1.2 μm, and particles of 2 μm and larger are collected with efficiencies of 75% and higher. Theoretical estimate of its d_{50} using Equation 3 yielded a value of 1.7 μm.

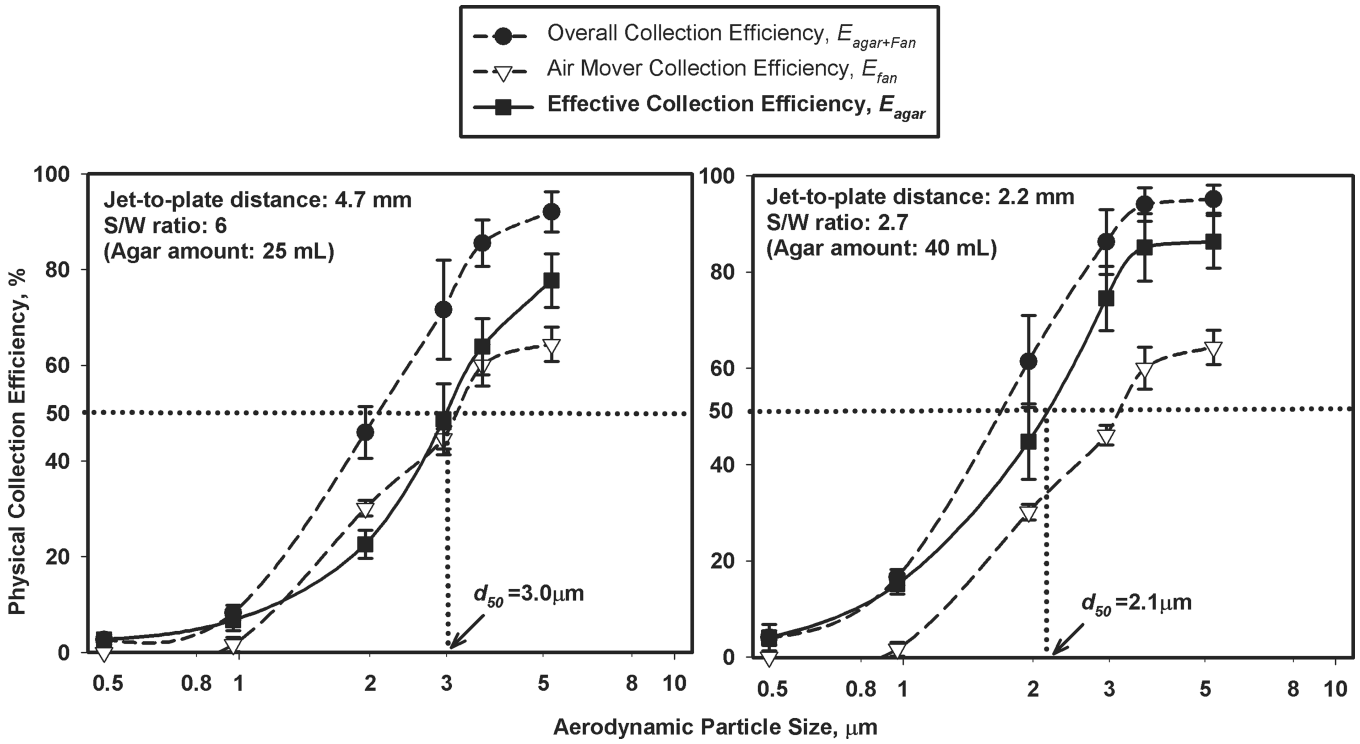

FIG. 6. Physical collection efficiencies of SAS Super 180 microbial sampler at different jet-to-plate distances. Data represent averages of three repeats and error bars stand for 1 standard deviation.

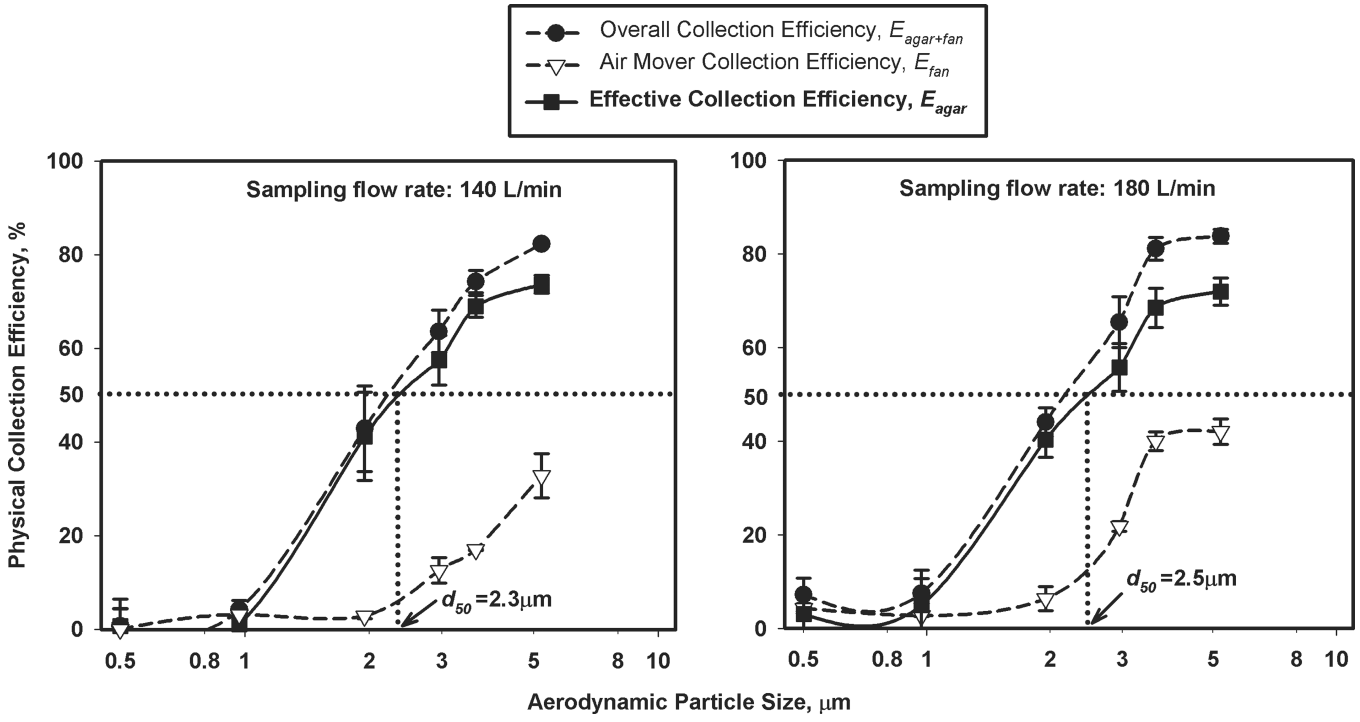

FIG. 7. Physical collection efficiencies of Millipore Air Tester at different sampling flow rates. Data represent averages of three repeats and error bars stand for 1 standard deviation.

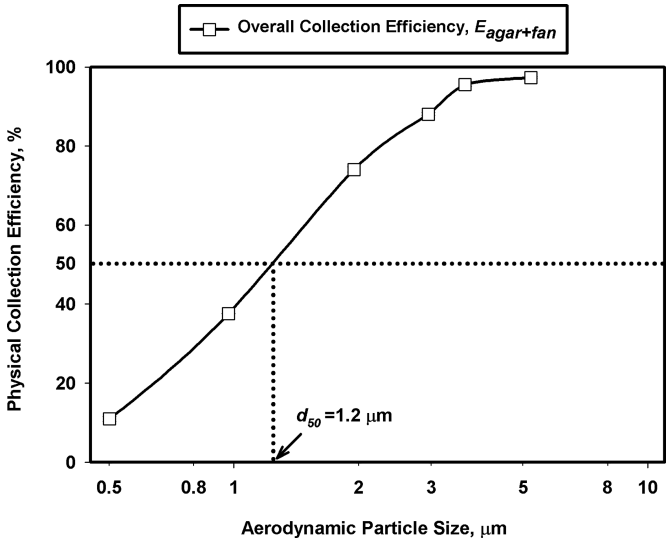

FIG. 8. Physical collection efficiency of RCS High Flow microbial sampler. Data represent averages from three repeats and error bars stand for 1 standard deviation.

The results presented above show experimental estimates of the collection efficiencies and cut-off sizes of seven portable microbial impactors. For most samplers, there was a difference between the calculated and the experimental cut-off size of the impactors as shown in Table 1. The following paragraphs discuss the impactor parameters that could have caused the differences between the theoretical and experimental cut-off sizes.

Jurcik and Wang (1995) indicated that there often are some discrepancies between the collection efficiency shapes determined experimentally and those calculated theoretically. The outcome of the theoretical estimate may depend on the grid spacing (Rader and Marple 1985), model geometry (Jurcik and Wang 1995) and other factors. Equation 1 used to calculate six samplers' theoretical d_{50} is designed for a single nozzle impactor. Most of the samplers investigated in this study, however, have multiple round nozzles, with the Millipore Air Tester having as many as 1,000 nozzles. Although it is generally assumed that calculations for a single-nozzle design can be directly applied to multiple nozzle impactors, some studies indicate that cross-flow from neighboring jets influences the particle collection efficiency (Sethuraman and Hickey 2001; Fang et al. 1991). Based on their studies with MOUDI impactor, Fang et al. (1991) recommended that their-derived cross-flow parameter should be below 1.2. Among the investigated portable samplers, the MAS-100 and the SAS Super 180 had cross-flow parameter of 1.06. The SMA impactor has a very low cross-flow value of 0.3. The cross-flow parameters for all other multi-nozzle samplers were above the recommended value of 1.2. In addition, the arrangement of multiple circle nozzle arrays may also play a role in the overall performance of the impactors by causing changes in the flow mechanics of the impaction region, the location of the jet stagnation points, and the impaction characteristics of each nozzle (Kwon, Kim, and Lee 2002).

The theoretical estimate of a single nozzle impactor by Rader and Marple (1985) indicates that S/W ratio from 1.0 to 5.0 has almost no effect on the impactor's $\sqrt{\text{Stk}_{50}}$. Our experimental data, however, indicate that the S/W value may play an important role in microbial impactor's performance. We observed that for the MAS-100 sampler, the cut-off size, d_{50}, decreased from 2.5 μm to 1.7 μm when the S/W ratio decreased from 9 to 4. The decrease in S/W also resulted in a steeper collection efficiency curve. Similar trend was observed for the SAS Super 180 sampler, where the cut-off size, d_{50}, decreased from 3.0 μm to 2.1 μm when S/W ratio decreased from 6 to 2.7. This change, again, was brought about by using 50 mL of agar instead of recommended 25 mL. For both samplers, when the S/W was decreased, the experimental d_{50} moved closer to the theoretical d_{50}. Kwon et al. (2002) also indicated in their study that increasing the S/W ratio from 1.75 to 2.25 decreased particle collection efficiency by more than 10%. We believe that in the investigated portable impactors' higher S/W ratios cause dissipation of the impaction jets, thus decreasing particle collection efficiency.

Another potential reason for the difference in the theoretical and experimental estimates of the impactors' d_{50} is potential particle bounce from the collection medium. The bounce of particles in the impactors is recognized as one of greatest limitations in their use (Hering 2001). We believe that the particle bounce was the reason for the increased d_{50} of the Millipore Air Tester when the sampling flow rate was increased from 140 to 180 L/min in the same sampling cycle.

Particle bounce from the agar collection medium and from the sampler's housing might also explain the flattening of collection efficiency curves of some samplers, e.g., MAS-100, SAS Super 180, and Millipore Air Tester, when collecting particles larger than 3 μm. Similar effect was observed with SMA Micro-Portable and BioCulture impactors when sampling 9.8 μm PSL particles. The flattening of the collection efficiency curve as a function of particle inertia ($\sqrt{\text{Stk}}$) when collecting particles on a variety of surfaces has been reported by Rao and Whitby (1978). Since agar is a semi-solid surface the effect is less pronounced than for uncoated glass surface or glass fiber filter. On the other hand, the hardening of agar due to desiccation by the impactor jets might increase the effect. The bounce of incoming particles off the already collected particles, especially when sampling particles of larger diameter, might have also contributed to the flattening of the collection curves. We believe these effects explain why the 100% collection efficiency was not observed in the devices tested.

For other impactors with investigated different sampling flow rates, the increase in the sampling flow rate resulted in a lower d_{50}, i.e., higher collection efficiency for smaller particles. These measurements at a higher sampling flow rates were performed using fresh agar plates. For example, the d_{50} of the SMA sampler decreased to 4.8 μm from a much higher value when the sampling flow rate was increased from 28.3 L/min to 141.5 L/min

as shown in Figure 2. However, the use of higher collection flow rate results in higher power consumption, which would shorten portable samplers' battery life. Higher flow rate also means more intense desiccation of agar which may lead to a lower recovery of the sensitive organisms.

The Reynolds number, Re, for most of the samplers was between 500 and 3000 in accordance with recommendations of Marple and Willeke (1976). The BioCulture and Microflow, however, had Re values of 200 which resulted in a less steep collection efficiency curve.

The theoretical d_{50} of the RCS High Flow was higher than the experimental value. The difference may be due to the contribution of impaction mechanism to the overall particle deposition on agar, while Equation (3) takes into account only centrifugal forces. The theoretical d_{50} for BioCulture was also higher than experimental value. The jet nozzles of this sampler have a shape of expanding cone and we used the inner nozzle diameter to calculate the theoretical d_{50}. It is likely that the jet was narrower than the inner nozzle diameter which resulted in a lower experimental d_{50}.

We believe that the interaction of the factors described above lead to a difference between theoretical and experimental cut-off sizes of the impactors.

As observed from their collection efficiency curves, the use of investigated samplers may result in a substantial underestimation of the bacterial concentration levels because their cut-off sizes, d_{50}, are above 1 μm, which is the size of most common individual bacteria. The bacterial aggregates or bacteria attached to larger particles, however, would be collected more efficiently. In addition, stress such as desiccation and impaction may further reduce the number of culturable bacteria recovered by the samplers. The common fungal particles are larger, usually 2–5 μm, therefore their collection would be efficient with most of the investigated samplers.

After anthrax incidents of 2001, many samplers have been suggested for the detection of the culturable *B. anthracis* cells and spores. *Bacillus anthracis* spores usually are rods with size of 1–1.5 μm by 3–10 μm (Friedlander 1997). Weis et al. (2002) collected viable *B. anthracis* spores in a contaminated US Senate Office using 6-stage Andersen impactor and reported that the majority of spores (70–90%) range from 0.65–2.0 μm in aerodynamic diameter with about 60% of spores in 1.1–2.0 μm range. Thus, samplers with the cut-off sizes above 2 μm would collect very few single *B. anthracis* spores. However, they should perform better if spore aggregates are involved. Thus, the users would be advised to check samplers' performance characteristics before their application for *B. anthracis* detection.

In general, based on our experimental results it appears that most of the portable samplers would provide only qualitative data when collecting individual bacteria. Some of the investigated samplers might be more efficient when collecting larger fungal spores. For more quantitative studies involving exposure to bacteria, robust bioaerosol samplers with cut-off sizes as low as 0.5 μm need to be developed. The results from this study may serve as a reference when selecting bioaerosol samplers for a particular application.

In addition to physical performance, stress on biological particles during their collection also plays a significant role in their enumeration. The biological performance of the portable samplers will be addressed in a separate study.

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