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Abstract

Exposure to airborne biological agents, especially to pathogenic or allergenic microorganisms, is known to cause a wide range of respiratory and other health disorders in occupational and general populations. Exposure to airborne microorganisms is commonly assessed by using air samplers designed for monitoring viable microbial agents. Recently, a number of new or modified portable samplers for viable microbial aerosols have become available, but their performance and accuracy was largely unknown. Accuracy of the data obtained using any bioaerosol sampler is of critical importance, because improper measurements may disguise actual differences in worker exposures and generally misguide the investigation. Thus, the main goal of this research was to analyze the physical and biological performances of newly available or modernized portable bioaerosol samplers, such as the 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). Their performance was analyzed theoretically and compared in laboratory and field experiments against a traditional reference bioaerosol sampler (Andersen-equivalent impactor) and an inhalation-based sampler (Button Aerosol Sampler with gelatin filter) for viable bioaerosols. In addition, portable samplers' physical collection efficiency was compared against the conventions for total inhalable particles and particle deposition in the lung.

The theoretical and experimental analysis of the portable samplers' physical collection efficiency indicated that cut-off sizes, or d_{50}, of the sampler is > 1μm, which would result in a substantial under-sampling of single bacterial cells with diameter 0.5-1.0 μm. It was found that the collection efficiency curves of all the samplers did not follow the inhalation exposure convention for bacterial particles, but some samplers did follow the convention for larger fungal particles. Some of the samplers' collection efficiency curves followed conventions for particle deposition in the lung. Laboratory investigation showed that the samplers' overall performance when sampling bacteria and fungi depended on sampler model and microorganism type collected, with their overall performance largely determined by the physical collection efficiency. Overall, the portable impactors underperformed compared to the traditional Andersen-equivalent impactor. Field experiments indoors and outdoors showed that relative performance of all the portable samplers was statistically different (lower) compared to the Andersen-equivalent impactor, except the RCS High Flow and the MAS-100 impactors. The recovery of microorganisms sampled on a gelatin filter was on average 50% of that recovered by the Andersen-equivalent impactor. Field investigation also revealed that the sampling time should be limited to a few minutes; otherwise the collected microorganisms may be inactivated due to desiccation.

The use of portable impactors is advantageous due to their high sampling flow rate, light weight and portability. However, this study showed that some of them underperform compared to a traditional Andersen-equivalent impactor and that the occupational professionals should be aware of the limitations of the portable samplers. The results from this study will help occupational professionals to select an efficient tool for environmental investigation projects; the results of the study and its recommendation will also be useful when designing new bioaerosol samplers that allow improved assessment of microbial exposure.

Highlights/Significant Findings

This research analyzed the physical and biological performances of newly available or modernized portable bioaerosol samplers. The analyzed samplers included the 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). Their performance was compared against a traditional reference bioaerosol sampler (Andersen-equivalent impactor) and an inhalation-based sampler (Button Aerosol Sampler with gelatin filter) for viable bioaerosols. Theoretical analysis, laboratory and field experiments were used to analyze whether the portable bioaerosol samplers could be used to accurately determine the levels of airborne viable bacteria and fungi. In addition, each sampler's physical efficiency was compared against the conventions for total inhalable particles and particle deposition in the lung.

Physical collection efficiencies of the portable bioaerosol samplers

Theoretical analysis of samplers' cut-off size, or d_{50} (size of particles at which 50% of them are collected), indicated that this parameter differs substantially for the investigated samplers. The theoretical d_{50} varied from ~1 μm for the Millipore Air Tester to as high as 17.5μm for the Microflow when operating at 30 L/min. The MAS-100 and the SAS-180 had theoretical d_{50} < 1.5μm, while the d_{50} for the RCS High Flow was ~1.7μm. The d_{50} of the SMA MicroPortable varied from 6 to 13.7 μm depending on the selected flowrate, while the BioCulture had a d_{50} of 8μm. The calculated d_{50} of the investigated samplers as well as other parameters are presented in Table 4.1.

Experimental collection efficiency of a sampler is often determined by directly comparing particle concentrations upstream and downstream of the sampler without considering the particle losses inside the sampler. Here, we developed a new approach which tests the collection efficiencies of the samplers with and without agar medium loaded. This method thus allows estimating the effective collection efficiency, i.e., the fraction of incoming particles deposited onto the agar collection medium only and the extent of losses inside the samplers. When experimentally testing samplers' d_{50} with polystyrene latex (PSL) particles, the determined values 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 Millipore Air Tester; 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 method that was develop in this research and yielded the effective collection efficiency. When collecting single cells of test bacteria, the RCS High Flow and the MAS-100 collected 20-30%, while other samplers collected less than 10% of these bio-particles. When sampling single spores of test fungi, the RCS High Flow and the SAS Super 180 deposited 80-90% of airborne spores on agar – highest among investigated samplers. Other samplers showed collection efficiencies of 10-60%.

Comparison of samplers' collection efficiency curves with particle inhalation convention curves showed that the RCS High Flow and the SAS Super 180 could be used to assess inhalation exposure to particles larger than 2.5 μm, such as fungal spores. Performance of the RCS High

Flow sampler was also reflective of the particle lung deposition pattern when sampling both bacteria and fungi. The MAS-100 and the SAS Super 180 matched the total deposition curve fairly well when collecting bacterial and fungi species, respectively. For other tested samplers we observed substantial differences between their collection efficiencies and lung deposition efficiencies for particles of corresponding size.

Relative biological efficiency of the portable samplers

The relative biological efficiencies of the portable impactors was determined by comparing their ability to recover culturable particles of three bacterial (*Pseudomonas fluorescens*, *Escherichia coli*, *Bacillus subtilis*) and three fungal (*Cladosporium cladosporioides*, *Penicillium melinii*, *Aspergillus versicolor*) species against that of an Andersen-equivalent type impactor (BioStage, by SKC Inc., Eighty Four, PA) and a Button Aerosol Sampler (SKC Inc.) equipped with gelatin filter. In order to determine the physical parameters governing the performance of portable impactors, their relative overall efficiency was correlated with their collection efficiency, jet velocity and jet-to-plate distance.

Experimental data indicated that performance of portable microbial samplers varied from model to model, and depended on the investigated bioaerosol species. When enumerating single bacterial cells, the BioStage impactor performed better than portable impactors and the relative overall performance of the impactors was highly correlated with their collection efficiency (adjusted R^2=0.74 - 0.94); inclusion of samplers' jet velocity and jet-to-plate distance into multiple liner regression did not affect the adjusted R^2 values. When enumerating single airborne fungal spores, the BioStage reference impactor also performed better than portable impactors, except for *C. cladosporioides* fungus, when the MAS-100 and SAS Super 180 performed equally well. Use of the collection efficiency in the linear regression as the sole predictor of the impactors' relative overall performance yielded adjusted R^2 values ranging from 0.61 to 0.73; inclusion of jet velocity and jet-to-plate distance into multiple liner regression increased the adjusted R^2 values to the range of 0.75 – 0.89. The data demonstrate that in addition to collection efficiency, jet velocity and jet-to-plate distance also play an important role in the enumeration of bioaerosols by microbial impactors.

When sampling using Button Aerosol Sampler with gelatine filter, the physical collection efficiency was approximately 100% for all six test microorganisms. However, we observed zero recovery of *P. fluorescens* and *E. coli* bacteria and lower recovery of *C. cladosporioides* fungal spores compared to the BioStage impactor. The recovery of *B. subtilis* bacteria, *A. versicolor* and *P. melinii* spores was not statistically different from that of the BioStage impactor.

Performance of samplers in a field environment

The performance of portable microbial samplers was investigated in indoor and outdoor environments when collecting bacteria and fungi and their performance was compared against that of the BioStage impactor and the Button Aerosol Sampler equipped with gelatin filter. The results showed the sampling environment can have a statistically significant effect on sampler performance, most likely due to the differences in airborne microorganism composition and/or their size distribution. The relative performance of the samplers also exhibited a day-to-day variation, most likely due to changes in airborne microorganism composition and/or their size distribution. Analysis of the data polled from all trials using ANOVA showed that relative

performance of all samplers was statistically different (lower) compared to the BioStage, except the RCS High Flow and the MAS-100 impactors. The MAS-100 also had statistically higher performance compared to other portable samplers, except the RCS High Flow. The Millipore Air Tester and the SMA had the lowest performances among the investigated samplers. It was also observed that performance of BioCulture and Microflow impactors which have $d_{50} > 7\mu m$ was better that could be predicted by the physical collection efficiency alone. It was likely due to the attachment of bacterial and fungal particles onto large particles which these samplers collected relatively efficiently and preserved their viability due to low impaction velocity.

The concentration of microorganisms collected by the gelatin filter was statistically significant (lower) compared to the BioStage impactor. On average, the microorganism concentration recovered on a gelatin filter was approximately 50% of that recovered by the BioStage impactor.

The relative performance of the investigated portable impactors was successfully described using a multiple linear regression model ($R^2 = 0.83$) and the effects of samplers' cutoff sizes and jet-to-plate distances as predictor variables were statistically significant. We believe that this empirical model (presented in Equation 7.3) could be used as an approximation in predicting overall performance of the portable microbial impactors when sampling in indoor and outdoor environments.

Effect of sampling time on performance of portable impactors

We analyzed whether the sampling time affects the overall performance of seven portable impactors mentioned above when collecting airborne bacteria and fungi from 2 to 30 min indoors and outdoors. In addition, to better ascertain the factors responsible for likely decrease in samplers' performance with prolonged sampling times, we separately investigated the effects of desiccation damage to the already collected microorganisms as well as the effect of agar desiccation prior to collecting the microbial particles. When bacteria were first collected by the impactors and then exposed to particle-free air 28 min, their average relative recovery decreased from 0.57 (particles collected for 2 min and not exposed to particle-free air) to 0.03 (particles collected for 2 min and then exposed to particle-free air for 28 min). For fungi, the situation was similar and the average relative recovery decreased from 0.3 to 0.03 under the same conditions. When the impactors collected particle-free air first and then collected bioaerosols for a fixed amount of time, the result was similar. The relative recovery for bacteria averaged for all samplers decreased from 0.77 (agar plates not exposed to particle-free air and particles collected for 2 min) to 0.04 (agar plates exposed to particle-free air for 28 min and then particles collected for 2 min). For fungi, the situation was similar and the relative recovery averaged for all samplers decreased from 0.4 to 0.02 under the same conditions as for bacteria.

The obtained data clearly indicate that recovery of airborne microorganisms collected using portable impactors as well as traditional stationary impactors decreases as the sampling time increases. Once the organisms are deposited onto the collection media they are being exposed to air flow over them as the sampling continues causing their dehydration and inactivation. If an area under the impaction nozzle is exposed to air prior to depositing the organism it becomes desiccated and hard resulting in a microorganism bounce or reduced access to nutrients. The effect of both of these factors was statistically significant.

Translation of Findings

To locate sources of biological contamination, to improve exposure assessment and control, to analyze the transmission of infectious diseases, to decrease the exposure risks and to protect the populations and resources potentially exposed to airborne microbial agents, advanced detection and sensing systems are needed for occupational health applications. Exposure to airborne microorganisms is commonly assessed by using air samplers designed for monitoring viable microbial aerosols (Macher, 1997). Recent advancements in sampling technology have produced a number of new or modified portable samplers that have not yet been thoroughly evaluated. Accuracy of the data obtained using any bioaerosol sampler is of critical importance, because improper measurements may disguise actual differences in worker exposures or introduce artificial ones (Macher, 1997). Underreporting of microbial concentrations due to poor performance of a sampler would indicate a low concentration of airborne microorganisms, when, in fact, people were exposed to much higher concentrations. Following a bioterrorism event, samplers of viable microorganisms are used to determine the extent and degree of contamination, to support the decisions regarding the need for medical treatment or cleanup, and to provide guidance whether the clean-up has been adequate.

Thus, the primary goal of this research was to investigate the physical and biological performances of newly available or modernized portable bioaerosol samplers and to compare their performance against established technology for sampling viable microorganisms, such as Andersen-type impactor. In addition, performance of portable impactors was compared against an inhalation-based sampler (Button Aerosol Sampler with gelatin filter). The information on the samplers' performance is expected to help in selecting an effective sampling tool for analyzing and solving a particular occupational health problem related to bioaerosol exposure. Information about the portable samplers' performance relative to a standard sampler (Andersen-equivalent impactor) should also aid in assessing the accuracy and validity of obtained concentrations of viable airborne microorganisms.

The experimentally measured physical collection efficiency data indicate that all of the investigated portable samplers have their cut-off size, or d_{50}, > 1μm which would result in a substantial under-sampling of bacterial particles, 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 bacterial agglomerates, larger fungal spores, or biological particles attached to larger particles. To collect particles of interest, field professionals should select a sampler that features a d_{50} that is sufficiently low to collect particles of interest.

After anthrax incidents of 2001, many bioaerosol samplers have been suggested for the detection of the culturable *Bacillus anthracis B. anthracis* cells and spores. *B. anthracis* spores usually are rods with size of 1-1.5 μm by 3-10 μm (Friedlander, 1997). The majority (70-90%) of viable *B. anthracis* spores collected in a contaminated US Senate Office using 6-stage Andersen impactor were reported to range from 0.65-2.0 μm in aerodynamic diameter with about 60 % of spores in 1.1-2.0 μm range (Weis et al., 2002). 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.

Based on the investigation of samplers' physical performance, none of the samplers could be used to estimate inhalation exposure of bacterial-sized particles because of the low collection efficiency. However, the RCS High Flow and the SAS Super 180 samplers could be used to assess inhalation exposure to particles larger than 2.5 μm, such as fungal spores. The RCS high flow could also be used to assess particle deposition in the lung, because its performance was reflective of the particle lung deposition pattern when sampling both bacteria and fungi. The MAS-100 and the SAS Super 180 matched the total deposition curve fairly well when collecting bacterial and fungi species, respectively, and could also be use to estimate particle deposition in the lung.

Analysis of the overall performance of the portable impactors (determined concentration of the microorganisms) in a laboratory relative to the BioStage impactor indicated that their performance is largely determined by their cut-off size. Overall, among the investigated portable samplers, the MAS-100 impactor seemed to perform the best, while the RCS High Flow and the SAS Super 180 often had somewhat lower relative efficiencies. The recovery of single bacteria and fungal spores by other impactors was substantially lower compared to the BioStage impactor. One has to note that the impactor efficiency was analyzed relative to the BioStage impactor. If the BioStage has a low biological performance when sampling sensitive bacteria, then even a high relative efficiency of a portable impactor does not indicate its high absolute biological performance.

When Button Aerosol Sampler with gelatine filter was used to collect viable bacteria and fungi for 5 min, no colonies of sensitive *P. fluorescens* and *E. coli* bacteria were observed; in addition, recovery of *C. cladosporioides* fungal spores was 20% compared to BioStage impactor. The result indicates the susceptibility of these microorganisms to desiccation stress and points to limitations of using gelatine filter as a collection medium for sensitive viable organisms. It could be used for the collection of hardy microorganisms, such as *B. subtilis*, recovery of which was not statistically different from the BioStage impactor.

The data from the field study showed that relative performance of all samplers was statistically different (lower) compared to the BioStage, except the RCS High Flow and the MAS-100. The data from field study indicated that impactors' relative performance is largely determined by their cut-off size, or d_{50}. However, other factors, such as jet-to-plate distance also seem to play a role. Thus, when selecting a tool for bioaerosol investigation, field professionals should consult the available literature to determine if the tool of their choice exhibits satisfactory performance. Again, to collect particles of interest, a sampler that features a d_{50} that is sufficiently low to collect particles of interest should be used.

The recovery of microorganisms on a gelatin filter was statistically significant (lower) compared to the BioStage impactor. On average, the microorganism concentration recovered on a gelatin filter was approximately 50% of that recovered by the BioStage impactor. This result shows that the gelatin filter may not be the best choice for sampling of viable microorganisms due to desiccation effects, especially if longer sampling times are required.

The investigation of sampling time effect showed that both the desiccation of already collected microorganisms as the sampling continues and well as desiccation of collection media prior to collecting microorganisms play a substantial and statistically significant role in decreased

number of measured microorganisms. Thus, it is suggested that that when impactors are used for the collection of airborne bacteria and fungi, sampling times should as short as reasonably possible to minimize under-representation of airborne microorganism concentration. Otherwise, use of longer sampling times would result in reduced recovery of collected microorganisms which would under-report and misrepresent the concentration of microorganisms in the air. Inaccurate data on airborne microorganism concentrations are likely to lead to erroneous conclusions of the investigation.

It is hoped that the data from this study will aid field professionals when selecting a portable sampler for environmental monitoring and when designing new microbial samplers for collection of culturable bioaerosols.

Outcomes/Relevance/Impact

The main findings of the study could be summarized as follows:

- The experimentally measured physical collection efficiency data indicate that all of the investigated portable samplers have their cut-off size, or d_{50}, > 1 μm, which would result in a substantial under-sampling of bacterial particles, especially of single bacterial cells with diameter 0.5-1.0 μm.
- None of the investigated portable samplers could be used to estimate inhalation exposure of bacterial-sized particles because of the low collection efficiency. However, some samplers could be used to assess inhalation exposure to particles larger than 2.5 μm, such as fungal spores. Also, physical collection efficiency of some samplers was reflective of the particle lung deposition pattern when sampling both bacteria and fungi.
- Overall performance of the portable samplers when sampling laboratory-generated bacteria and fungi was primarily determined by their physical collection efficiencies, i.e., low physical collection efficiencies yielded low overall performance and high physical collection efficiencies yielded high overall performance.
- Use of gelatin filter in a laboratory study yielded low recovery of sensitive microbial species compared to the BioStage impactor (equivalent to Andersen impactor).
- In a field environment, the sampling time when using portable impactors should be as short as reasonably possible. Otherwise, the recovery of the collected microorganisms will be reduced due to desiccation of already collected microorganisms as well as the hardening of collection medium.
- Based on the field sampling data, the relative performance of all samplers was statistically different (lower) compared to the BioStage impactor (equivalent to Andersen impactor), except the RCS High Flow and the MAS-100 samplers. The data from field study indicated that impactors' relative performance is mostly determined by their cut-off size, or d_{50}. However, other factors, such as jet-to-plate distance also seem to play a statistically significant role.
- In the field study, the recovery of microorganisms on a gelatin filter was statistically significant (lower) compared to the BioStage impactor. On average, the microorganism concentration recovered on a gelatin filter was approximately 50% of that recovered by the BioStage impactor

Given the data presented in this report, the field professionals should be aware of the limitations of the portable samplers when selecting a tool for their investigations. Use of a poorly performing sampler may result in an underreporting of microbial concentrations in an occupational environment. Since samplers of viable microorganisms are often used to determine the extent and degree of exposure or contamination, and to support decisions regarding the need for medical treatment or cleanup after exposure to bioaerosols, representative data obtained by such samplers is of high importance. Thus, selection of a proper tool could be especially critical.

Therefore, as one potential outcome of the study, it is recommended that field professionals reference this and similar studies on performance of bioaerosol sampling tool to make an informed decision regarding the sampler used in their investigations. Since the performance of a portable impactor seems to be largely determined by its cut-off size, samplers that feature d_{50} that is sufficiently low to collect particles of interest should be used. In addition, sampling time should be as short as reasonably possible to minimize potential reduction in viability of collected biological particles. It would also be recommended to refer not only to studies listing the samplers' cut-off sizes, but also their overall performance relative to other biological samplers. This will allow to better gauge the performance of the sampler and will help evaluating accuracy of the collected data.

Another potential outcome of the study is recommendation for future research. The portable impactors obviously offer certain advantages over stationary samplers: high sampling flow rate, light weight and portability. However, the results of this study and demonstrated shortcomings of portable samplers point to new directions in research to address these shortcomings:

- Since the performance of portable impactors seems to be largely determined by their cut-off sizes, their cut-off sizes should be improved (lowered) to improve collection of single bacteria, i.e., the cut-sizes should be 0.5μm or less.
- To improve our ability to assess exposure (either inhalation exposure or assessment of particles deposited in the lung), sampler performance should be mimic health-based conventions for inhalable, thoracic and respirable bioaerosols; or total particle deposition in the lung. Ideally, one sampler could be adjusted to perform according to several sampling conventions. Data obtained by such samplers would contribute to building dose-response relationships relevant to biological aerosols.
- Short sampling time remains one of the critical issues when sampling viable biological aerosols. Samplers should be developed that enable longer sampling times. This would enable averaging airborne concentrations over time periods longer than few minutes and would provide more accurate exposure assessment data, especially where long-term exposures are concerned.
- Current inhalable samplers (such as Button Aerosol Sampler) hold a great promise to be used as samplers for viable biological aerosols; however the lack of media capable of preserving the viability of collected particles remains their primary limitation. As was shown in this research, use of gelatin filter allowed the recovery of only 50% viable particles concentration compared to and Andersen-equivalent impactor. Thus, one of the goals of future research could be development of sampling media compatible with existing inhalable samplers while capable of preserving viability of collected microorganisms.

Another important outcome of this project, which responded to PA-01-032 "Career Development Grants in Occupational Safety and Health Research", was professional development of the PI. This Career Grant in Occupational Safety and Health provided the PI with a unique opportunity to acquire greater experience and expand skills as a researcher while gaining the necessary research and grant management expertise. This research project gave the PI a great opportunity to work independently and improve his ability to achieve research goals, and to manage a multi-year grant. Such experience was of great value when developing as an independent scientist. In addition, the project allowed a unique opportunity to work with undergraduate and graduate students and help in their professional development. . In addition, it played a substantial role in one student's Ph. D. thesis. This research project resulted in four published peer-reviewed manuscripts, one manuscript in preparation and numerous presentations at National and international conferences and workshops. The process of preparing the manuscripts, responding to Reviewer comments and critique offered a great opportunity to develop as an independent researcher. I strongly believe that by using my skills acquired through the Career Grant and by conducting further research in occupational and environmental health I will be able to contribute towards improvement of occupational conditions for American workers.

Scientific Report/Research Results

1 Background

This project responded to PA-01-032 "Career Development Grants in Occupational Safety and Health Research". The research performed under this project evaluated newly available or modernized portable bioaerosol samplers in the laboratory and field settings and determined their applicability for monitoring viable airborne bacteria and fungi. The biological performance of portable samplers was compared against an Andersen-equivalent impactor and an inhalation-based sampler adapted for viable bioaerosols. This proposal also responded to NORA's Priority Research Areas: Infectious Diseases, Indoor Environment, and Exposure Assessment Methods.

Many processes generate bioaerosols of diverse forms ranging from submicron allergens to the larger bacteria, fungi, and pollen. Exposure to airborne biological agents, especially to pathogenic or allergenic microorganisms, may cause a wide range of respiratory and other health disorders in occupational and general populations. Health-care professionals increasingly recognize bioaerosols as a cause of preventable airborne infections and hypersensitivity diseases (WHO, 1990). It is estimated that 250 million episodes per year of respiratory infection in the US are attributed to bioaerosol exposure. They result in 75 million physician visits per year and 150 million days lost from work with medical care costs of ca. $10 billion plus loss of income of ca. $10 billion (Cox and Wathes, 1995). Moreover, there is an increased threat that biological warfare agents, such as anthrax-causing *Bacillus anthracis* spores, may be used by terrorists and could be released against civil/governmental/military establishments. As anthrax incidents of Fall of 2001 have shown, contamination of occupational facilities with *B. anthracis* spores causes not only significant work disruption and economic loss, but, more importantly, poses fatal risks to present workers.

Exposure to biological aerosols is encountered in numerous occupational and residential environments. Bacterial infections, microbial allergy and toxicoses caused by exposure to

bioaerosols in agriculture and in the food processing industry have been reported (Cox and Wathes, 1995). One study concluded that high concentrations of organic dust in agriculture are common and that the concentrations of gram-negative bacteria in swine confinements and compost facilities are frequently in the tens of thousands per m^3 (Clark, 1986). High microbial concentration have been reported in hatcheries and other agricultural facilities (Morring, 1989),; during silo uncapping (May et al., 1989) and unloading (Morey et al., 1989); in mushroom culturing environments and in cow barns (Lacey and Crook, 1988). Farmer's lung disease is a form of allergic alveolitis caused mainly by the inhalation of spores of the thermophilic actinomycete *Faenia rectivirgula* which may be present in large numbers in moldy and badly stored hay (Lacey et al., 1972). Inhaling organic dusts containing fungal and actinomycete spores can induce allergic rhinitis, chronic bronchitis, extrinsic allergic alveolitis, and organic toxic dust syndrome (Lacey and Crook, 1988).

Various illnesses and infections due to microbiological exposures have been found in metal working fluid environments (Robertson et al., 1988; Popendorf et al., 1996; Graves et al., 1997; Kriebel et al., 1997; Robins et al., 1997; Kennedy et al., 1999), textile manufacturing industries (Schachter et al., 1984), solid waste treatment facilities (Lembke and Kniseley, 1980; Rahkonen, 1990) and food processing industries (Cox and Wathes, 1995). Exposure to bioaerosols is also of concern in health-care environments. Although encountered concentrations are usually much lower than those in agricultural settings (Yeh et al., 1995), the pollutant can disperse among immunocompromised individuals which signifies the exposure response issue. Organisms that are aerosolized during surgery may transmit blood-borne pathogens from the infected patients to the health care workers. Exposure of workers to infectious diseases is also a concern. It is estimated that 5.3 million workers are exposed to tuberculosis at work in the US (ACGIH, 1997).

Biocontamination of indoor air environments, including both occupational and residential spaces, is gaining increased attention because of the adverse health effects such as asthma and other allergic diseases (Burge, 1990; Miller, 1992; Spengler et al., 1993; Koskinen et al., 1995). It was estimated that about 36% of residential homes in US have mold problems (Spengler et al., 1993). A health hazard evaluation by indicated that indoor air quality investigations have increased from 2% in 1980 to 40 % in recent years and that many of these complaints are related to airborne biological particles (NIOSH, 1997).

To locate sources of biological contamination, to improve exposure assessment and control, to analyze the transmission of infectious diseases, to decrease the exposure risks and to protect the populations and resources potentially exposed to airborne microbial agents, advanced detection and sensing systems are needed for occupational health applications. Exposure to airborne microorganisms is commonly assessed by using air samplers designed for monitoring viable microbial aerosols (Macher, 1997). A considerable number of bioaerosol samplers are available, ranging from simple non-quantitative devices such as settling plates for species identification to sophisticated instruments such as the direct-reading Ultraviolet Aerodynamic Particle Spectrometer (UVAPS, TSI Inc., St. Paul, MN). Such devices collect airborne microorganisms onto agar plates by impaction, into a liquid by impingement, or onto a dry, non-nutrient surface by filtration.

In general, the performance of aerosol sampling devices is characterized by their physical efficiency: ability to aspirate particles into the sampler's inlet, to transmit them through the sampler's interior and to collect them onto the collection surface (Grinshpun et al., 1994). In the case of viable microbial sampling, the sampler's biological performance, or its ability to maintain microbial viability, also needs to be evaluated (Thompson et al., 1994). This makes the evaluation more complicated (Kang and Frank, 1989), as viable airborne particles cannot, in general, be recovered without some inactivation or loss during or after sampling (Burge and Solomon, 1987; Eduard et al., 1990); (Jensen et al., 1992; Buttner and Stetzenbach, 1993; Buttner et al., 1997). For quantitative exposure assessments, high collection efficiency is desirable; however, very often microorganism inactivation necessitates accepting a tradeoff between the collection efficiency and the viability of the microorganisms (Stewart et al., 1995). The collection efficiency and microbial recovery of commonly used bioaerosol samplers depend on particle cut-off size, the inlet efficiency, the amount of drying during and after collection, the collection flow rate and the surface density of the collected microorganisms. Comparative studies in the field and the laboratory have shown considerable differences among the numbers of airborne microorganisms measured with different sampling devices (Delmore and Thompson, 1981; Jensen et al., 1992). A thorough review of the performance of bioaerosol samplers introduced prior to 1994 has been provided (Henningson and Ahlberg, 1994). However, since that time many new samplers have been introduced into the market.

Many bioaerosol samplers require external vacuum source, which usually limits investigator's mobility. This limitation can be countered through the use of portable samplers (Mark et al., 1995). The lightweight portable samplers do not require heavy and noisy vacuum pumps and could be used where electricity is not available, or is hazardous. Some of these portable samplers have been included in bioaerosol field studies to collect bacteria and fungi (Jensen et al., 1992; Mehta et al., 1996; Mehta, 2000; Bellin and Schillinger, 2001). However, recent advancements in sampling technology have produced a number of new or modified portable samplers that have not yet been thoroughly evaluated. Accuracy of the data obtained using any bioaerosol sampler is of critical importance, because improper measurements may disguise actual differences in worker exposures or introduce artificial ones (Macher, 1997). Underreporting of microbial concentrations may result in erroneous conclusions about the exposure levels. Following a bioterrorism event, samplers of viable microorganisms are used to determine the extent and degree of contamination, to support the decisions regarding the need for medical treatment or cleanup, and to provide guidance whether the clean-up has been adequate (MMWR, 2001). An underperforming sampler may indicate adequate cleanup, while, in fact, cleanup is still not adequate.

Therefore, it is necessary to investigate the performance of any bioaerosol sampler before it can be used for exposure assessment or to address other occupational health problems. In addition, most of the samplers designed to collect and recover biological particles do not necessarily follow conventions for inhalable, thoracic and respirable fractions as defined by (CEN, 1993; ISO, 1995; ACGIH, 1999). These conventions describe particle penetration into the overall respiratory system (inhalable aerosol), into the tracheobroncheal system (thoracic aerosol) and into the alveolar region (respirable aerosol) as a function of particle aerodynamic diameter, with thoracic and respirable aerosol as subfractions of the inhalable aerosol (Maynard and Jensen, 2001). For example, a convention for inhalable aerosols shows that particles smaller than 1 μm

penetrate into the respiratory system with nearly 100% efficiency, while particles between 1 and 10 μm penetrate with efficiency between 80-100%. Various personal and stationary samplers designed to meet these criteria have been widely used to evaluate exposure to non-biological aerosols in workplace, as summarized by Maynard and Jensen (2001). Application of samplers that collect particles according to inhalation conventions would also be advantageous for assessing exposure to biological aerosols that will deposit in one or more regions in the lung (Griffiths et al., 1997). There has been some interest in adapting available inhalable samplers, such as the IOM (Institute of Occupational Medicine) sampler (SKC Inc., Eighty Four, PA), for size-selective bioaerosol sampling (Kenny et al., 1998). Such a modified sampler was used in the field to collect viable and total airborne microorganisms on size-fractionating foams (Kenny et al., 1999). Collected viable microorganisms were subjected to desiccation by continuing air flow which eliminated sampler's use for quantitative microorganism analyses by culture-based methods. Another sampler with the potential for size-selective bioaerosol sampling is the Button Aerosol Sampler (SKC Inc., Eighty Four, PA). This sampler collects particles on a filter with high degree of uniformity and meets several "ideal bioaerosol sampler" requirements (Macher, 1997): size-selective sampling, low sensitivity to wind direction and velocity, and ease of handling (Aizenberg et al., 1998). This sampler was used to enumerate total airborne spores and it was found suitable for sampling of total bioaerosols (Aizenberg, 2000). Application of this sampler for viable bioaerosols indicated that bacterial vegetative cells quickly loose their viability, primarily due to desiccation stress (Wang, 2001). Compared to commonly used filters, such as MCE or polycarbonate, gelatin filters retain moisture and, thus, may significantly improve survival of viable cells. In light of these findings, it is possible that the Button Aerosol Sampler could be modified and adapted for collection of viable biological aerosols. If successful, a validated Button sampler could be recommended to serve as a reference sampler when evaluating portable samplers for viable bioaerosols.

In the performed research, we evaluated the physical and biological performances of seven portable bioaerosol samplers when collecting airborne bacteria and fungi. These test samplers have either recently been introduced into the market or have been modified to collect at higher flowrates or without the need for proprietary media. Manufacturers of most of the test samplers claim that their products could be used to collect spores of *B. anthracis*. This claim was verified by using a non-pathogenic *B. anthracis* simulant as one of the test microorganisms.

The initial evaluation of the portable bioaerosol samplers was performed under controlled laboratory conditions so that extraneous variables, such as variability of the microorganisms sampled, the general aerosol size distribution and the sampling conditions could be controlled (Jensen et al., 1992). Following the laboratory experiments, the samplers were evaluated in different field environments. Field evaluations can provide valuable additional information, because sampler performance in the field may not always agree with the predictions based on laboratory assessments (Macher, 1997).

2 Objectives and Specific Aims

In this research we analyzed the physical and biological efficiencies of newly available or modernized portable bioaerosol samplers. Their performance was compared against a traditional reference bioaerosol sampler (Andersen-equivalent impactor) and an inhalation-based sampler

for viable bioaerosols. Through the theoretical analysis, laboratory and field experiments we determined whether these samplers can be used to accurately determine the levels of airborne viable bacteria and fungi. In addition, each sampler's physical performance was compared against the conventions for total inhalable particles and particle deposition in the lung. The information on the samplers' performance is expected to help in selecting a proper sampling tool for analyzing and solving a particular occupational health problem related to bioaerosol exposure.

The research objectives were achieved through the following Specific Aims:

I. Development of the experimental system for testing the physical and biological efficiencies of portable bioaerosol samplers
II. Theoretical analysis of test bioaerosol samplers
III. Determination of the physical collection efficiency of the portable samplers when challenged with non-biological and biological test particles
IV. Evaluation of physical and biological efficiency of the inhalable reference sampler for viable bioaerosols
V. Evaluation of the biological performance of the portable bioaerosol samplers
VI. Investigation of the sampling time effect on the performance of the samplers
VII. Field evaluation of the portable bioaerosol samplers
VIII. Summary of the results

The Procedures, Methodology, Results and Discussion described below are organized as separate chapters that correspond to one or more Specific Aims.

3 Specific Aim I: *Development of the experimental system for testing the physical and biological efficiencies of portable bioaerosol samplers*

3.1 Selection of test and reference samplers

We evaluated the physical and biological performances of seven portable bioaerosol samplers that have recently been introduced or modified to sample at higher flowrates or eliminate the need for proprietary collection media. Most of these samplers employ mechanism of impaction as a primary collection mechanism; however they differ in design, jet-to-plate distance and collection flow rate. We hypothesized that due to these differences the test samplers will differ in physical and biological performance. As suggested by (Liden, 1994), to evaluate the performance of different air samplers, they must be compared with another sampler. The Andersen impactor six-stage viable bioaerosol impactor (Graseby Andersen, Atlanta, GA) has long been regarded as the sampler of choice for enumerating the viable airborne microorganisms (Jensen et al., 1992) and has been recommended and used as a reference sampler in several bioaerosol studies (Jones et al., 1985; Chatigny et al., 1989). It is also an instrument recommended by NIOSH Method 0800 Bioaerosol Sampling (NIOSH, 1994). Therefore, we used this sampler as a reference sampler in our study. However, it was estimated that Andersen sampler's entrance efficiency (product of the aspiration and the transmission efficiency) and found that the entrance efficiency may exceed 100% (oversampling) for particles larger than 1 μm if the sampler faces the wind; the entrance efficiency maybe be significantly less than 100%

(undersampling) if the sampler is placed upright at 90° to the wind direction (Grinshpun et al., 1994). The deviation increases with increasing particle size and wind velocity. Similar findings were reported by an experimental study which also concluded that sampler's performance bears no relation to health-related criteria (Upton et al., 1994).

Therefore, to reflect current understanding of health-based aerosol sampling, we included a second reference sampler, which has been shown to follow inhalation convention when sampling aerosol particles. As discussed in the Background section, the Button Aerosol Sampler (SKC, Inc.) was found to follow convention for inhalable aerosols and has been applied to enumerate viable and total microorganisms (Aizenberg et al., 1998; Aizenberg et al., 2000; Wang et al., 2001). While enumeration of total spores was satisfactory, the bacterial vegetative cells quickly lost their viability, primarily due to desiccation stress. We believed that the biological efficiency of this sampler could be significantly improved by using gelatin filters instead of regular filters, such as polycarbonate. However, before this sampler could be used as reference for portable bioaerosol samplers, its performance when collecting viable particles had to be evaluated. Therefore the entire Specific Aim IV will be dedicated for evaluating this inhalable sampler when collecting test bioaerosols on gelatin filters. The test and reference samplers evaluated in this study are listed in Table 1. All these samplers (except the Button Aerosol Sampler) are impactors collecting particles onto a single agar plate. The RCS High Flow sampler collects particles onto proprietary agar strips supplied by the manufacturer instead of agar plates.

Table 3.1. The reference and test bioaerosol samplers evaluated in this study.

Bioaerosol Sampler	Manufacturer	Sampling flow rate, L/min	Sampling medium
Reference Samplers			
BioStage Impactor (Andersen impactor equivalent)	SLC Inc., Eighty Four, PA	28.3	Agar
Button Aerosol Sampler	SKC, Inc., Eighty Four, PA	4	25 mm filter
Test Samplers			
RCS High Flow	Biotest Diagnostics Corporation, Denville, NJ	100	Agar
SAS Super 180	Bioscience International, Rockville, MD	180	Agar
MAS 100 (Microbiological Air Sampler)	EMD Chemicals, Gibbstown, NJ	100	Agar
BioCulture	A.P. BUCK Inc., Orlando, FL	120	Agar
SMA MicroPortable.	Veltec Associates, Inc. Phoenixville, PA	28.3, 141.5	Agar
MicroFlow	Aquaria srl, Lacchiarella, Italy	30 - 120	Agar
Millipore Air Tester	Millipore Corp., Billerica, MA	150/180	Agar

3.2 Experimental setup

The experimental setup used to determine the physical and biological collection efficiencies of the test samplers is shown in Figure 3.1. The Collison six-jet nebulizer was used to aerosolize the non-biological and biological test 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 aerosolized particles passed through the Po-210 charge neutralizer and were then carried into the test chamber by the dry air flow Q_{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 30cm/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_{OPC} = 1.2 L/min.

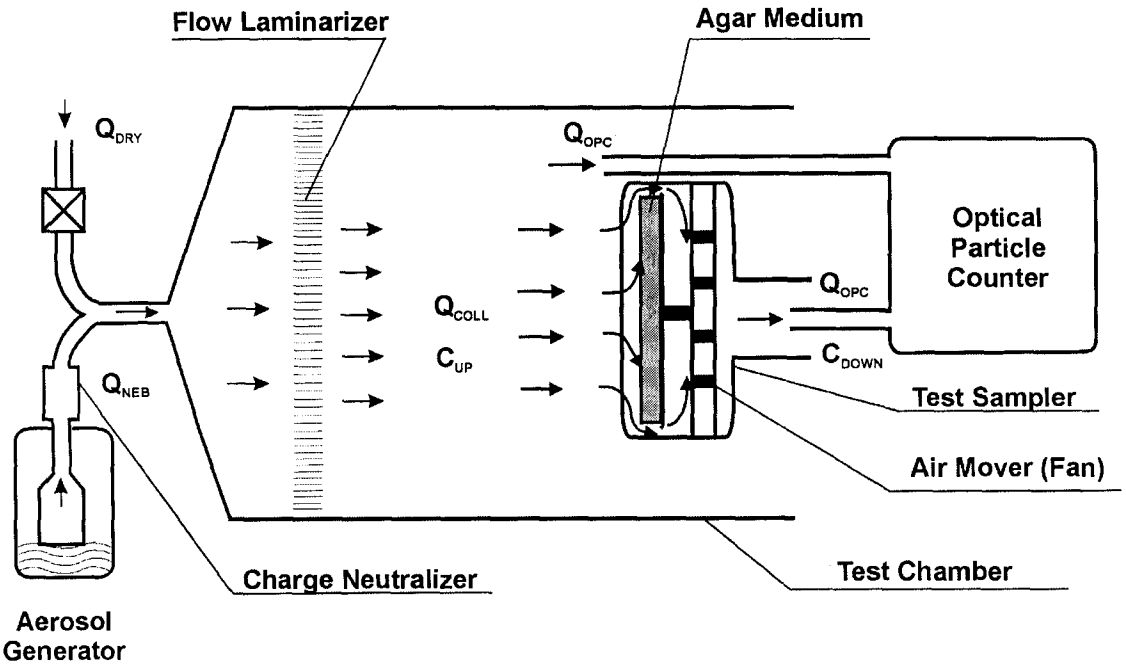

Figure 3.1. A schematic representation of the experimental setup used to analyze the performance of the portable samplers.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

The setup used for the experiments is slightly different than the one originally proposed, where a test sampler was to be operated in parallel to two reference samplers. The set up was modified once it was realized that to place a test sampler and two reference samplers side by side required a large chamber which presented difficulties achieving a uniform particle distribution across the chamber. The particle concentration in the chamber used for the experiments was uniform across the chamber within 5%. When testing biological performance of the portable impactors, the test and reference samplers were placed in the chamber in a randomized order while maintaining the same microorganism concentration in the test chamber.

4 Specific Aim II: *Theoretical analysis of test bioaerosol samplers*

The physical characteristics of the test 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 4.1.

The dominant parameter describing the collection efficiency of an impactor is the Stokes number. The recommended square root value of Stokes number corresponding to cut-off size (size of particle resulting in collection efficiency of 50%), or 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):

$$d_{50} = \sqrt{\frac{9\eta W}{\rho_p U_0 C_c}} \sqrt{Stk_{50}}, \qquad (4.1)$$

where η is the air viscosity, W is the diameter of the impactor nozzle, $\sqrt{Stk_{50}}$ 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:

$$V_T = \frac{C_C d^2 \rho_p V_i^2}{18\eta R}, \qquad (4.2)$$

where C_C is slip correction factor, V_i is the inlet velocity into centrifugal drum, η is the air viscosity, 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:

$$d_{50} = \sqrt{\frac{9\eta D}{2\pi N_e C_c V_i \rho_p}} \qquad (4.3)$$

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).

The calculated theoretical d_{50} of the investigated samplers is presented in Table 4.1. As could be seen, the theoretical d_{50} of the samplers varies from ~1 μm for the Millipore Air Tester to as high as 17.5μm for the Microflow when operating at 30 L/min. The MAS-100 and the SAS-180 had theoretical $d_{50} < 1.5$μm, while the d_{50} for the RCS High Flow was ~1.7μm.

Equation 4.1 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 (Fang et al., 1991; Sethuraman and Hickey, 2001). Based on their studies with MOUDI impactor, Fang et al. (1991) recommended that their-derived cross-flow parameter should be below 1.2. We also calculated this parameter for the investigated samplers and the results are presented in Table 4.1. 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.

The next chapter compares the experimentally obtained values of d_{50} with theoretical calculations and examines potential reasons for observed discrepancies.

Table 4.1. 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	28.3 141.5	12 12	6.3 6.3	9.3 9.3	0.3	25 25	5 5	0.8 0.8	528 2638	13.7 6.0	>10 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	100 100	400 400	0.7 0.7	3.1 3.1	1.06	50 30	2.8 6.4	4 9	503 503	1.47 1.47	1.7 2.5
Microflow	30 120	378 378	1.1 (outer diameter) 2.5 (inner diameter)	3.1 3.1	3.4	25 25	1.89 1.89	0.84 0.84	45-180 (for inner diameter)	17.5 8.7 (for inner diameter)	>10 8.8
SAS Super 180	180 180	401 401	0.8 0.8	2.4 2.4	1.06	40 25	2.16 4.7	2.7 6	791 791	1.3 1.3	2.1 3.0
Millipore Air Tester	140 180	1000 1000	0.46 0.46	0.8 0.8	1.7	Plate supplied by manufac-turer	5.84 5.84	12.7 12.7	423 544	1 0.9	2.3 2.5
RCS High Flow	100	N/A	N/A	N/A	N/A	Agar strip supplied by manufac-turer	N/A	N/A	N/A	~1.7	1.2

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

5 Specific Aim III: *Determination of the physical collection efficiency of the portable samplers when challenged with non-biological and biological test particles*

5.1 Introduction

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 0.65μm, while an AGI-30 impinger has a cut-off size of 0.3μm (RTI, 2004). Such cut-off sizes allow these samplers to collect most of the airborne single bacterial cells and fungal spores. The main goal of this part of the study was to investigate the physical collection efficiencies of test portable microbial samplers, to determine their cut-off sizes and to compare these values with theoretical estimates described above. The testing was performed with non-biological and biological particles. 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).

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

Another way to perform health-relevant bioaerosol sampling could be to determine not just the amount of inhaled particles, but the amount of particles deposited in the human respiratory system. In this case, the sampler's physical performance should mimic either particle deposition in different regions of the respiratory system or overall particle deposition which is defined as the combined deposition of particles in all regions of the respiratory system including head airways, tracheobronchial, and alveolar regions (Hinds, 1999). Certain instruments, such as different collection stages of Andersen impactor have been found to represent particle deposition in various regions of the lung (Rhodes et al., 2001). Based on the International Commission on Radiological Protection deposition model, the total particle deposition efficiency in the

respiratory system is a complex function of particle size and the lung geometry (ICRP, 1994). Correlation of total particle deposition efficiency in the lung with the collection efficiency of a bioaerosol sampler for microorganisms of certain size would provide more accurate estimates of actual human exposure to the airborne biological agents. However, very little information is available about whether the portable microbial samplers could be used to determine the amount of culturable bioaerosols inhaled or deposited in the lung.

Therefore, when testing with biological agents, the objectives of this part of the study were not only to investigate the collection efficiency curves of several portable microbial samplers, but also to determine whether these samplers could be used to determine the amount of microorganisms that are inhaled or deposited in human respiratory system. The latter objective was accomplished by comparing the samplers' collection efficiency curves with particle inhalation and deposition conventions for the human lung. Evaluations were performed using three bacterial and three fungal species.

5.2 Non-biological test particles

Polystyrene Latex (PSL) particles (Bangs Laboratories, Inc., Fishers, IN) were used as non-biological particles 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.

5.3 Biological test particles

The performances of the samplers were tested with three bacterial and three fungal species with aerodynamic sizes ranging from 0.6 to 3.1 μm. The bacterial species included vegetative cells of *Bacillus subtilis, Pseudomonas fluorescens* and *Escherichia coli. Bacillus subtilis* is a representative of hardy organisms and also an anthrax surrogate (Hill et al., 1999). *P. fluorescens* bacteria are commonly found in the ambient air environments (Nevalainen, 1989; Gorny et al., 1999) and are representatives of sensitive bacteria. *E. coli* bacteria have been suggested as one of the standards when testing bioaerosol sampling technologies (Macher, 1997). A recent study implicated airborne spread of *E. coli* O157 during outbreak investigation (Varma et al., 2003). *Pseudomonas fluorescens* and *Escherichia coli* were found present and posing a health concern in the floodwater following the Katrina disaster in New Orleans, LA (Presley et al., 2005). The tested fungal species included *Cladosporium cladosporioides, Aspergillus versicolor,* and *Penicillium melinii. C. cladosporioides* is one of the dominant fungal species in the air (Cooley et al., 1998). *A. versicolor* and *P. melinii* are believed to belong to the fungi genera that are capable of producing high level of toxic chemicals (Ciegler, 1978). Thus, the selected microorganism species represent sensitive and hardy bacterial species and a surrogate of a bio-threat agent as well as toxic fungal species that are commonly found in natural environments.

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

melinii (ATCC 10469) were grown on Malt Extract Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) for 1-2 weeks at room temperature.

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

5.4 Determination of the physical 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 3.1. 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 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:

$$E_{COLL} = (1 - \frac{C_{DOWN}}{C_{UP}}) \times 100\%, \qquad (5.1)$$

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

$$E_{agar+fan} = (1 - [(1 - E_{agar})(1 - E_{fan})]) \times 100\%, \qquad (5.2)$$

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:

$$E_{agar} = (1 - \frac{1 - E_{agar+fan}}{1 - E_{fan}}) \times 100\%. \qquad (5.3)$$

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 4% 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 to propagate the errors, which is shown in all figures involving the Effective Collection Efficiency.

5.5 Particle inhalation and deposition convention

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

$$IF(d_a) = 0.5(1+\exp(-0.06d_a)) \text{ for } U_0 < 4\text{m/s} \quad (5.4)$$

$$IF(d_a, U_0) = 0.5(1+\exp(-0.06d_a)) + 10^{-5}U_0^{2.75}\exp(0.055d_a) \text{ for } U_0 > 4\text{m/s}, \quad (5.5)$$

where d_a is the particle aerodynamic diameter in μm and U_0 is ambient air velocity. Equation (5.4) simulates indoor environments with air velocity less than 4 m/s, while Equation (5.5) simulates outdoor environments with air velocity higher than 4 m/s. The inhalation curve based on these equations defines the desired sampling performance of any inhalable particle sampler. Once a particle is inhaled, it can deposit inside the respiratory system due to the impaction, settling or diffusion mechanism. The International Commission on Radiological Protection developed a model that takes into account the level of activity, gender, breathing rate and frequency, and other parameters (ICRP, 1994). Hinds (1999) obtained simplified equation for the total particle deposition efficiency in the lung for monodisperse spheres of standard density at standard conditions:

$$DF(d_a) = IF(d_a)(0.0587 + \frac{0.911}{1+\exp(4.77+1.485\ln d_a)} + \frac{0.943}{1+\exp(0.508-2.58\ln d_a)}) \quad (5.6)$$

where $DF(d_a)$ is the total particle deposition efficiency, $IF(d_a)$ is inhalation fraction calculated by Equation (5.4) or (5.5), and d_a is the aerodynamic particle size. Equation (5.6) calculates the average particle deposition inside the respiratory system for men and women over three exercise levels. The equations above were used to determine the portable samplers' conformity to the particle inhalation and deposition conventions.

5.6 Samplers' performance when sampling non-biological particles

The experiments have 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 5.1. The theoretical estimates of the cut-off size of the sampler using the Equation 5.1 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, 1998).

Tests with BioCulture microbial sampler at 120 L/min showed that the d_{50} was approximately 7 μm as shown in Figure 5.2. 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 5.3. 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 experimental d_{50} in this case was 2.5 μm (Figure 5.3), 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 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 5.3. 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 is shown in Figure 5.4. The sampler's experimental d_{50} was 8.8 μm, which agreed well with the theoretical estimate of 8.7μm. 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 2.5mm. 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 120L/min this sampler seems to collect very few particles smaller than 3μm. Such performance would lead to undersampling of most environmental bacteria and fungi.

The data with SAS Super 180 are shown in Figure 5.5. 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 5.5. Overall, the effective collection efficiency of this sampler for the smaller jet-to-plate distance was above 80% for the particles 3μm 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 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 5.6. 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 5.6, 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 5.6, 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 5.7. 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 5.3 yielded a value of 1.7 μm.

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 4.1. The following paragraphs discuss the impactor parameters that could have caused the differences between the theoretical and experimental cut-off sizes.

It was indicated that there often are some discrepancies between the collection efficiency shapes determined experimentally and those calculated theoretically (Jurcik and Wang, 1995). 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 5.1 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 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 $\sqrt{Stk_{50}}$. 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}. One study also indicated that increasing the S/W ratio from 1.75 to 2.25 decreased particle collection efficiency by more than 10% (Kwon et al., 2002). 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 3μm. Similar effect was observed with SMA MicroPortable and BioCulture impactors when sampling 9.8 μm PSL particles. The flattening of the collection efficiency curve as a function of particle inertia ($\sqrt{Stk}$) 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 5.1. 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 (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 5.3 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 resulting in a lower experimental d_{50}.

5.7 Samplers' performance when sampling biological particles

5.7.1 Bioaerosol size distribution

Six species of bacteria and fungi were aerosolized to test the collection efficiencies of portable microbial samplers. These microorganisms ranged from 0.61 to 3.14 μm in mean aerodynamic size as shown in Figure 5.8. *Pseudomonas fluorescens* bacteria were the smallest with a mean aerodynamic size of 0.61 μm, and *Penicillium melinii* fungi were the largest particles with a mean aerodynamic size of 3.14 μm. For *E. coli, B. subtilis, C. cladosporioides,* and *A. versicolor,* the mean aerodynamic sizes were 0.78, 0.86, 2.53, and 2.89 μm, respectively. For *C. cladosporioides* and *A. versicolor*, the median aerodynamic size was about 4-8% larger than the mean aerodynamic, while for other microorganisms the mean aerodynamic size was approximately the same as median aerodynamic size. As seen in Figure 5.8, the size distributions of all microorganisms, except *C. cladosporioides,* feature one distinct peak reflecting their mean aerodynamic size. The size distribution of this particular microorganism had two peaks: the peak associated with smaller size was most likely due to the presence of fungal and mycelia fragments produced during the aerosolization process while the right-hand peak represented spores. The mean aerodynamic size of *C. cladosporioides* was calculated based on the peak with higher aerodynamic size. For other organisms, small peaks to the left of the main peak, as in the case of the *B. subtilis*, is likely the contribution of the culture medium. In the case of fungi, it is likely the contribution of mycelia fragments.

5.7.2 Samplers' collection efficiencies

The Overall Collection Efficiencies of the test samplers, $E_{agar+fan}$, were determined using Equation (5.2) and are shown in Figure 5.9. In general, the Overall Collection Efficiency of each sampler increased with increasing microorganism aerodynamic size. When collecting bacterial aerosols of *P. fluorescens, E. coli,* and *B. subtilis*, the Overall Collection Efficiency tended to be rather low for all the samplers tested. For *B. subtilis* aerosols, an anthrax-causing microorganism stimulant, MAS-100 performed best with about 40% Overall Collection Efficiency, while RCS High Flow had about 30% Overall Collection Efficiency. For the other five remaining samplers, the $E_{agar+fan}$ was less than 10% for all three bacterial aerosols tested.

When sampling fungal spores of *C. cladosporioides, A. versicolor, P.* and *melinii,* each tested sampler had similar overall collection efficiency for all three fungal species. Microflow had the lowest Overall Collection Efficiency for fungal particles, while SAS Super 180 had the highest Overall Collection Efficiency and efficiency values increased in the following order: Microflow, BioCulture, SMA, Millipore Air Tester, MAS-100, RCS High Flow, and SAS Super 180. MAS-100, RCS High Flow, and SAS Super 180 had the Overall Collection Efficiency above 80% when sampling fungal spores larger than 2.5μm. When sampling fungal spores the Overall Collection Efficiencies were less than 30% for BioCulture and for Microflow, approximately 50% for SMA, and 70% for Millipore Air Tester. As seen from Figure 5.9, SAS Super 180 performed best with fungal spores with $E_{agar+fan}$ of approximately 90%, while RCS High Flow and MAS-100 had slightly lower Overall Collection Efficiencies.

The Effective Collection Efficiencies, i.e. the proportion of microorganisms collected onto agar media of the tested samplers are shown in Figure 5.10. As explained above, the effective collection efficiency of the RCS High Flow is represented by the sampler's Overall Collection Efficiency. As shown in Figure 5.10, RCS High Flow had the highest Effective Collection Efficiency, approximately 90%, when collecting fungal spores larger than 2.5μm; SAS Super 180 had the second highest Effective Collection Efficiency – approximately 80%. Millipore Air Tester and MAS-100 had Effective Collection Efficiencies of approximately 60% when sampling fungal spores. For other samplers - SMA, BioCulture and Microflow, the E_{agar} was at 10% level for fungal aerosols tested.

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

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

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

Comparison of Figures 5.9 and 5.10 indicates that high Overall Collection Efficiency does not necessarily predict higher Effective Collection Efficiency. For example, the Overall Collection Efficiency of SMA when sampling *P. melinii* is about 50%, while its Effective Collection Efficiency is approximately 10%. Such a difference implies the possibility of the substantial particle losses inside the sampler. Particle losses for the tested samplers are described below.

We calculated each sampler's sampling loss as a difference between the Overall Collection Efficiency, $E_{agar+fan}$, and Effective Collection Efficiency, E_{agar}. The losses indicate the percentage of the microorganisms that were collected not on the collection medium, but elsewhere in the sampler. As shown in Figure 5.11, the highest sampling losses, approximately 40-50%, were observed for the SMA sampler, when sampling fungal spores. Microflow, MAS-100 and BioCulture had sampling losses of 20-30% for fungi and lower losses when sampling

bacteria. All other samplers had sampling losses at 10% level. SAS Super 180 had relatively high Effective Collection Efficiency and relatively low sampling loss. The extent of such losses, we believe, depends on the positioning and design of the air mover.

5.7.3 Samplers' conformance to health-relevant sampling conventions

The samplers evaluated in this study were developed for determining the presence of biological aerosols in various air environments. If samplers' performances simulate the particle inhalation or deposition efficiency in human lung, they would provide more relevant information related to the microbial exposure. Such information, along with relevant health symptoms, could be used to establish dose-response relationships related to bioaerosol exposure. The inhalable airborne particles can be characterized as the particles originally in the volume of air that are inhaled and enter the nose or mouth (Hinds, 1999). The inhalation convention curve described by Equation 5.4 for indoor environments is shown in Figure 5.12 as a solid line. The fraction of inhalable particles decreases with increasing particle size. From Figure 5.12 we can see that this fraction is 90% or higher for particles smaller than 3.5 μm, indicating that all six tested microbial particles will be inhaled with efficiency of 90% or higher. As observed from Figure 5.12, when sampling bacterial species, all tested samplers were found to have substantially lower Effective Collection Efficiencies compared to the inhalation curve, thus significantly under-sampling the bacterial species. When sampling larger particles, i.e. fungal spores larger than 2.5 μm, RCS High Flow and SAS Super 180 matched the inhalation curve with a difference of less than 10% for RCS High Flow and 15% for SAS Super 180. Millipore Air Tester had a difference of approximately 35% compared with the inhalation curve when sampling fungal particles. For other samplers, the difference was as large as 90% in comparison to the inhalation curve. These results indicate that RCS High Flow and SAS Super 180 are able to reflect the particle inhalation convention fairly well when sampling fungal species, while other samplers substantially undersample the fungal particles compared with inhalation convention.

An understanding of particle deposition efficiency in the human respiratory system as a function of particle size provides valuable information needed to adequately assess the health hazard as a result of inhalation of airborne particles. According to the fitted ICRP model for average particle deposition in male and female lungs under three different levels of activity- sitting, light exercise, and heavy exercise, the particle deposition efficiency for particles between 0.1 and 0.25μm decreases with increasing aerodynamic size (Hinds, 1999). For particles larger than 0.25μm and smaller than 10μm, the deposition efficiency increases with increasing particle size (Hinds, 1999). For particles larger than 10μm, the deposition fraction decreases with increasing particle size (Hinds, 1999). The total deposition of particles in the respiratory system within the investigated particle size range is calculated using Equation (5.6) and shown in Figure 5.12. As seen from the figure, RCS High Flow sampler is able to reflect the particle deposition patterns in the lung when sampling both bacteria and fungi, while MAS-100 and SAS Super 180 seem to reflect the particle lung deposition patterns for bacterial and fungi species, respectively. The total deposition fraction is about 35% for the *E. coli* aerosol compared with 22% Effective Collection Efficiency of MAS-100 sampler. There is a difference of less than 10% for RCS High Flow and SAS Super 180 between the total particle deposition and effective collection efficiency for three fungal spores tested ranging from 2.5 to 3.1 μm in aerodynamic size. Effective Collection Efficiencies of other investigated samplers do not seem to reflect the particle deposition pattern in the respiratory system. To address the particle health hazard, both particle inhalability and

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

The data presented in Figures 5.9-5.12 are based on physical performance of the samplers, i.e., particle removal from the air and their deposition on a culture medium. This evaluation does not take into account the biological performance of the samplers, i.e., the effect of sampling on the culturability of microorganisms. The extent of such effect would vary depending on the species in question and sampler design and is addressed later.

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

5.8 Discussion

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). The majority (70-90%) of viable *B. anthracis* spores collected in a contaminated US Senate Office using 6-stage Andersen impactor were reported to range from 0.65-2.0 μm in aerodynamic diameter with about 60 % of spores in 1.1-2.0 μm range (Weis et al., 2002). 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. In fact, all tested samplers were found to have substantially lower Effective Collection Efficiencies compared to the inhalation curve. Some of the investigated samplers might be more efficient when collecting larger fungal spores. For example, the RCS High Flow and the SAS Super 180 are able to reflect the particle inhalation convention fairly well when sampling fungal species, while other samplers substantially undersample the fungal particles compared with inhalation convention. 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 can serve as a reference when selecting bioaerosol samplers for a particular application.

An understanding of particle deposition efficiency in the human respiratory system as a function of particle size provides valuable information needed to adequately assess the health hazard as a result of inhalation of airborne particles. Our data indicate that the RCS High Flow sampler is able to reflect the particle deposition patterns in the lung when sampling both bacteria and fungi, while MAS-100 and SAS Super 180 seem to reflect the particle lung deposition patterns for bacterial and fungi species, respectively.

The data presented above are based on physical performance of the samplers, i.e., particle removal from the air and their deposition on a culture medium. This evaluation does not take into account the biological performance of the samplers, i.e., the effect of sampling on the culturability of microorganisms. The extent of such effect would vary depending on the species in question and sampler design. The biological performance of the portable samplers is addressed in the following chapter.

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

5.9 Figures for Chapter 5

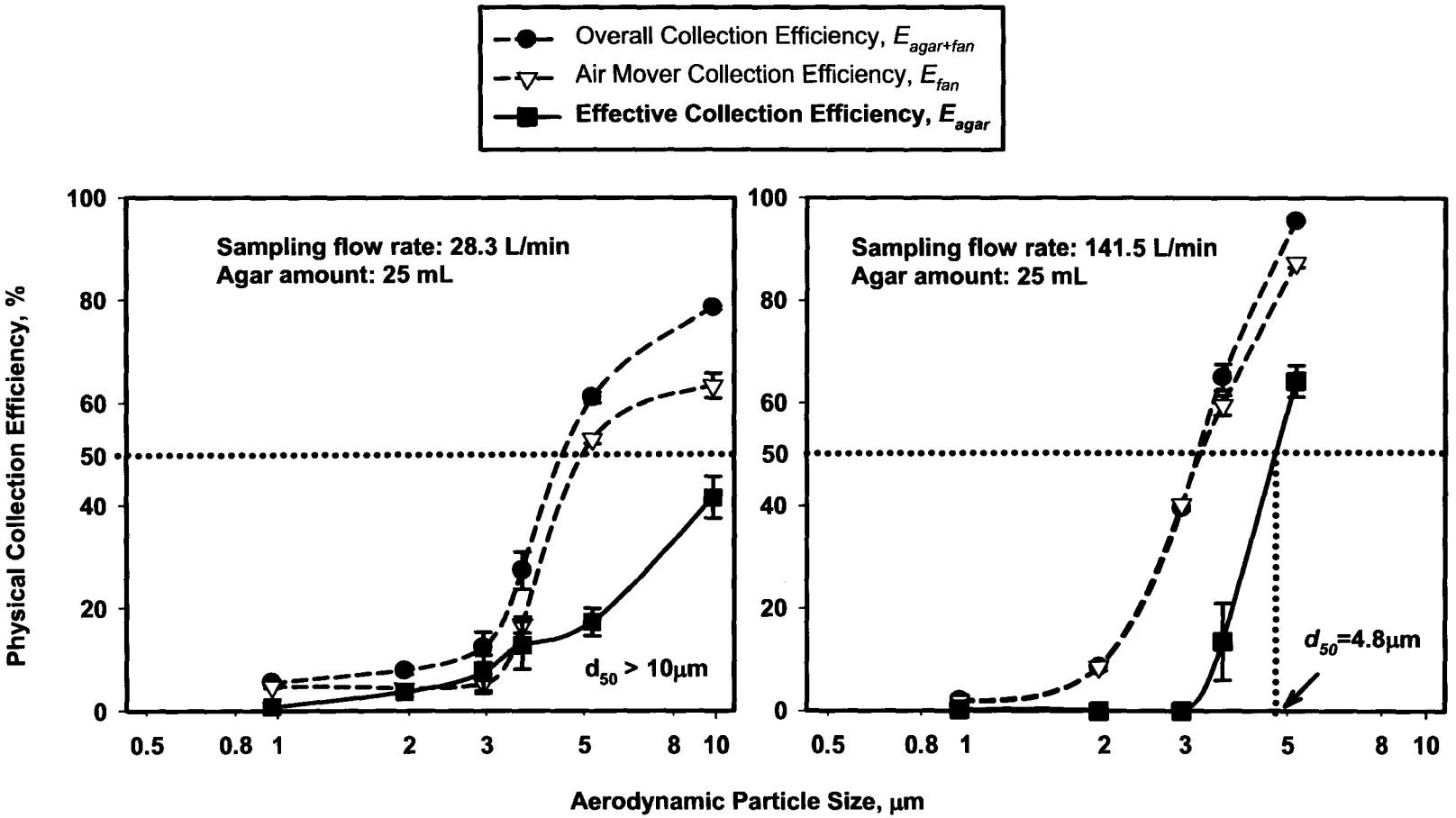

Figure 5.1. Physical collection efficiencies of SMA MicroPortable microbial sampler at different sampling conditions. Data represent averages of three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

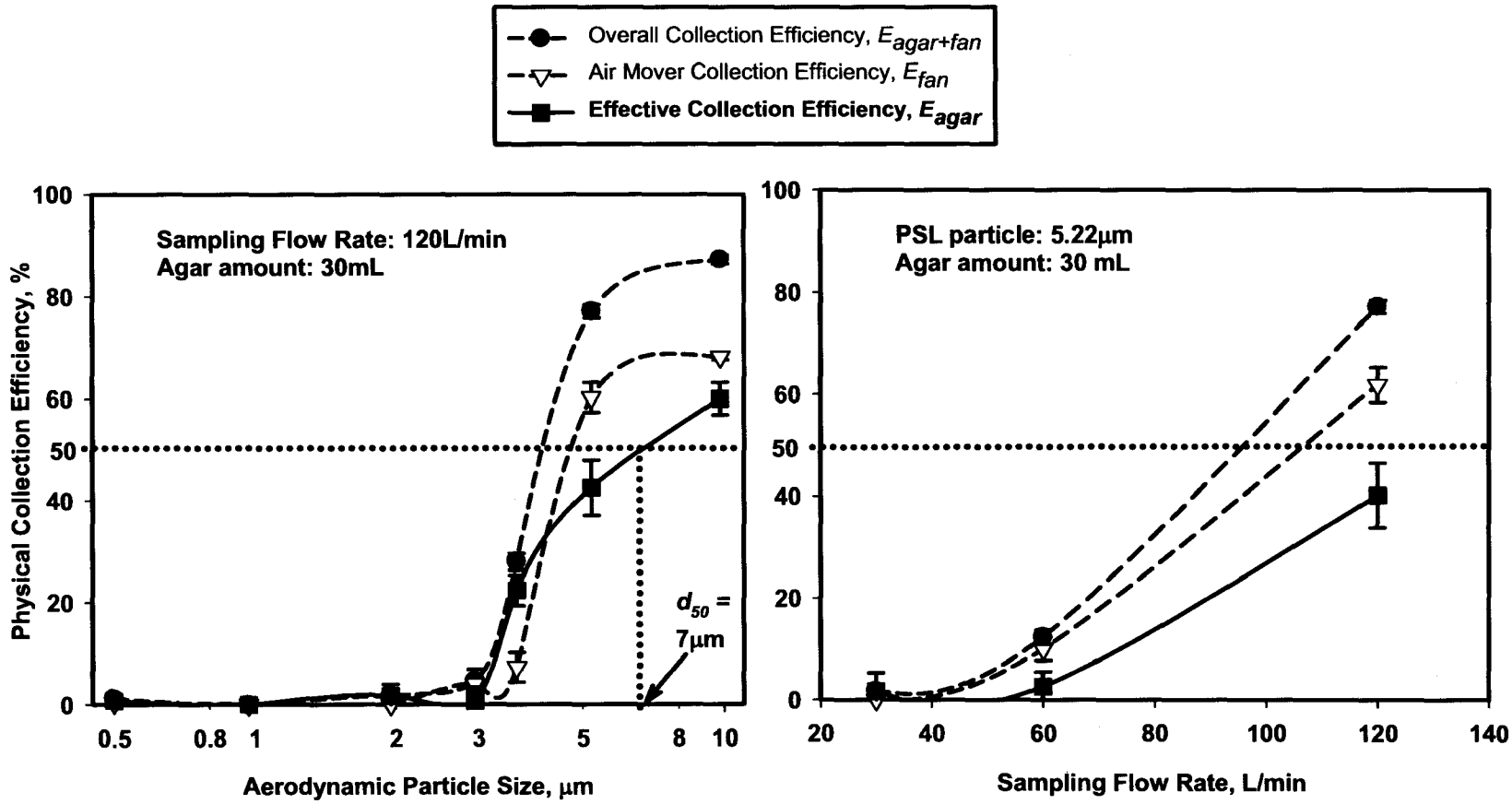

Figure 5.2. Physical collection efficiencies of BioCulture microbial sampler at different sampling conditions. The data represent averages from three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

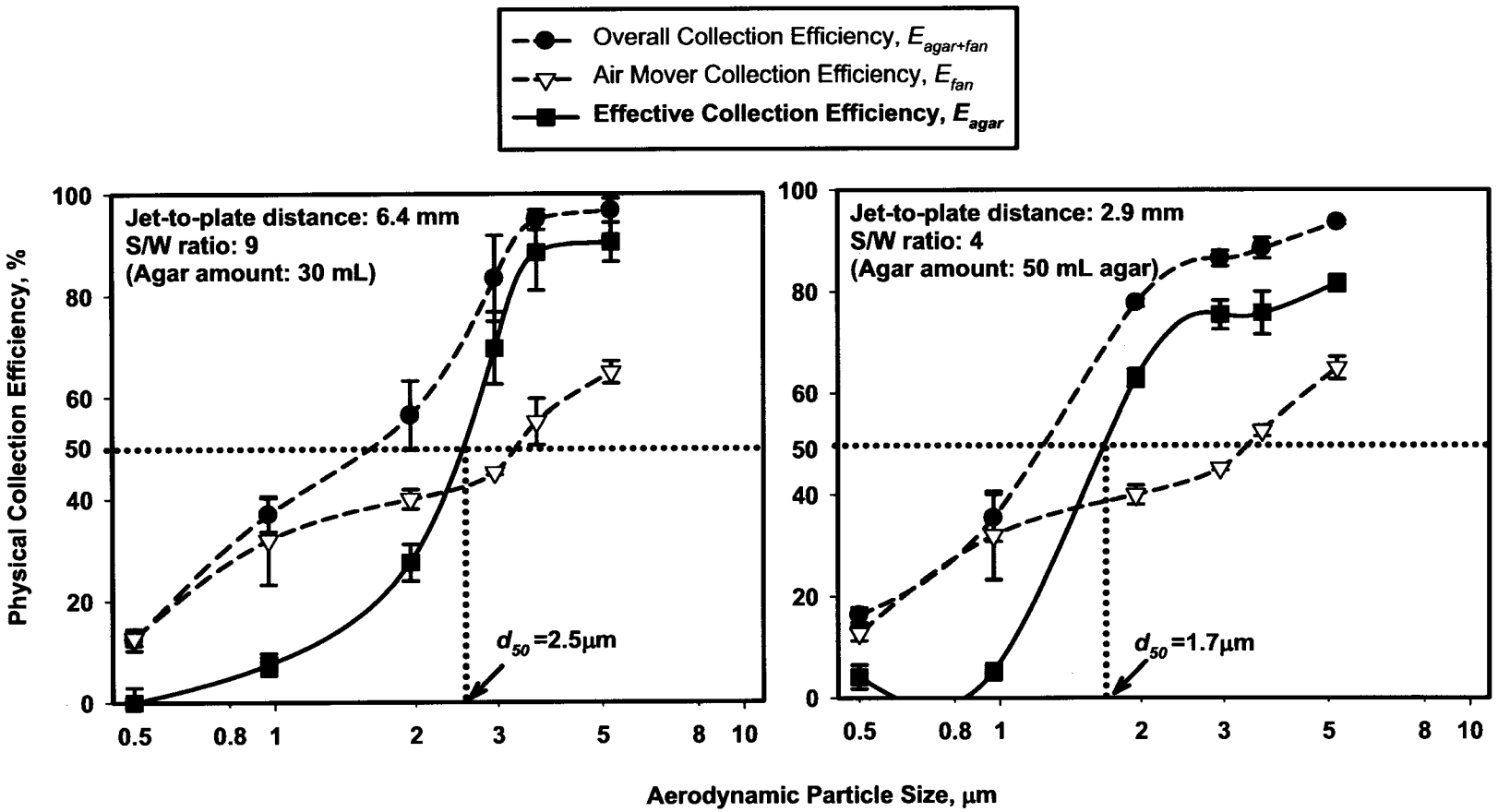

Figure 5.3. Physical collection efficiencies of MAS-100 microbial sampler at different jet-to-plate distances. The data represent averages from three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

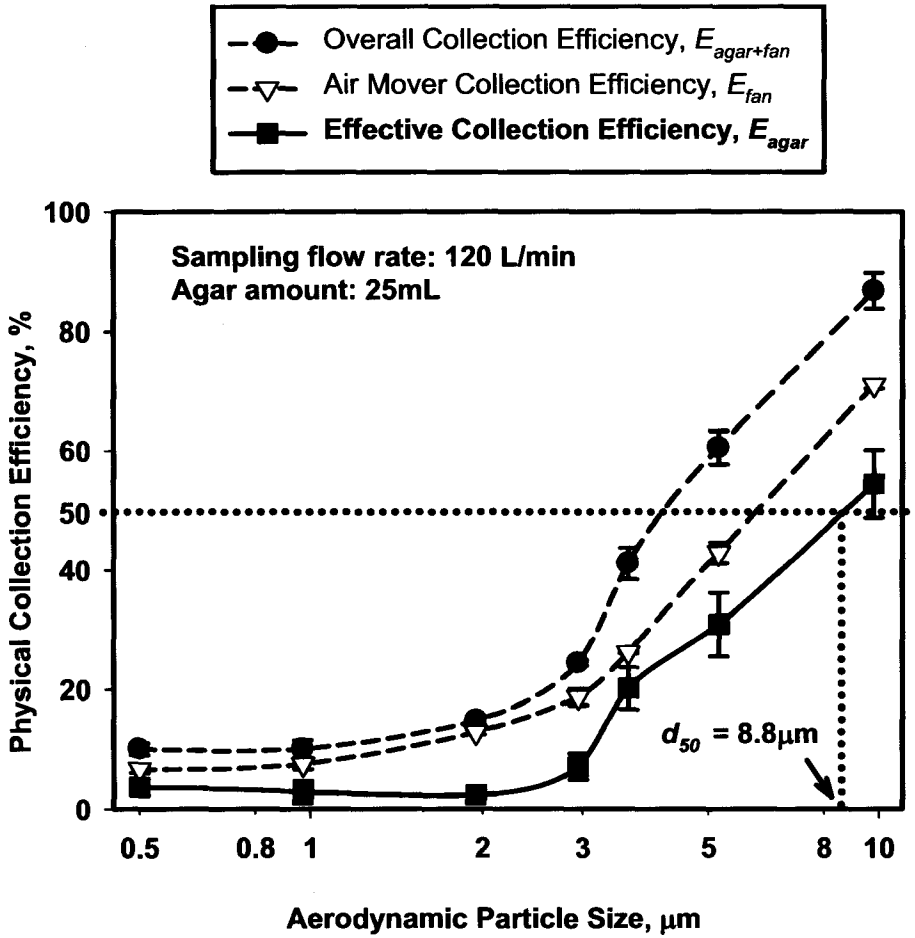

Figure 5.4. Physical collection efficiencies of Microflow microbial sampler. Data represent averages of three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

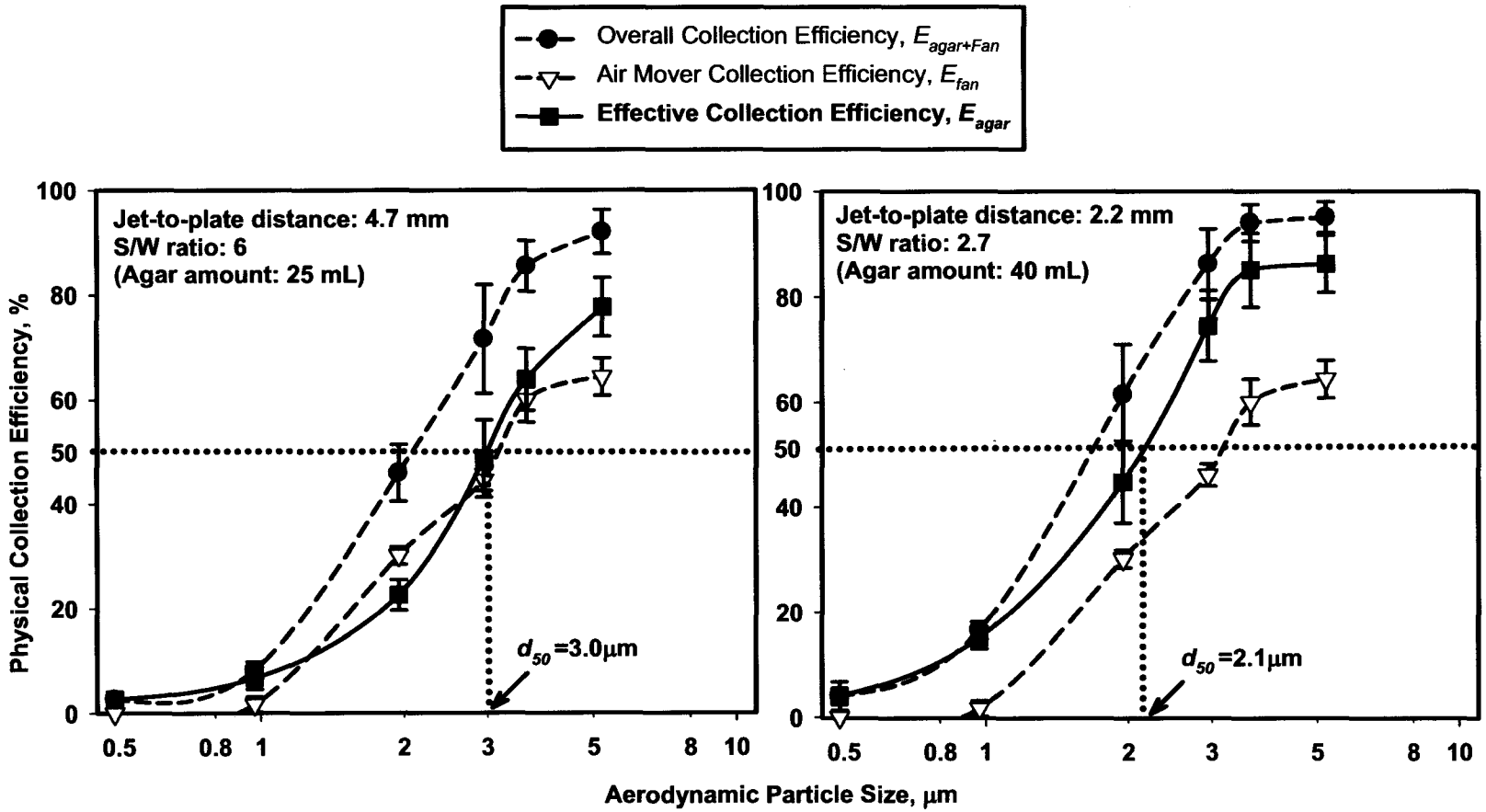

Figure 5.5. Physical collection efficiencies of SAS Super 180 microbial sampler at different jet-to-plate distances. Data represent averages of three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

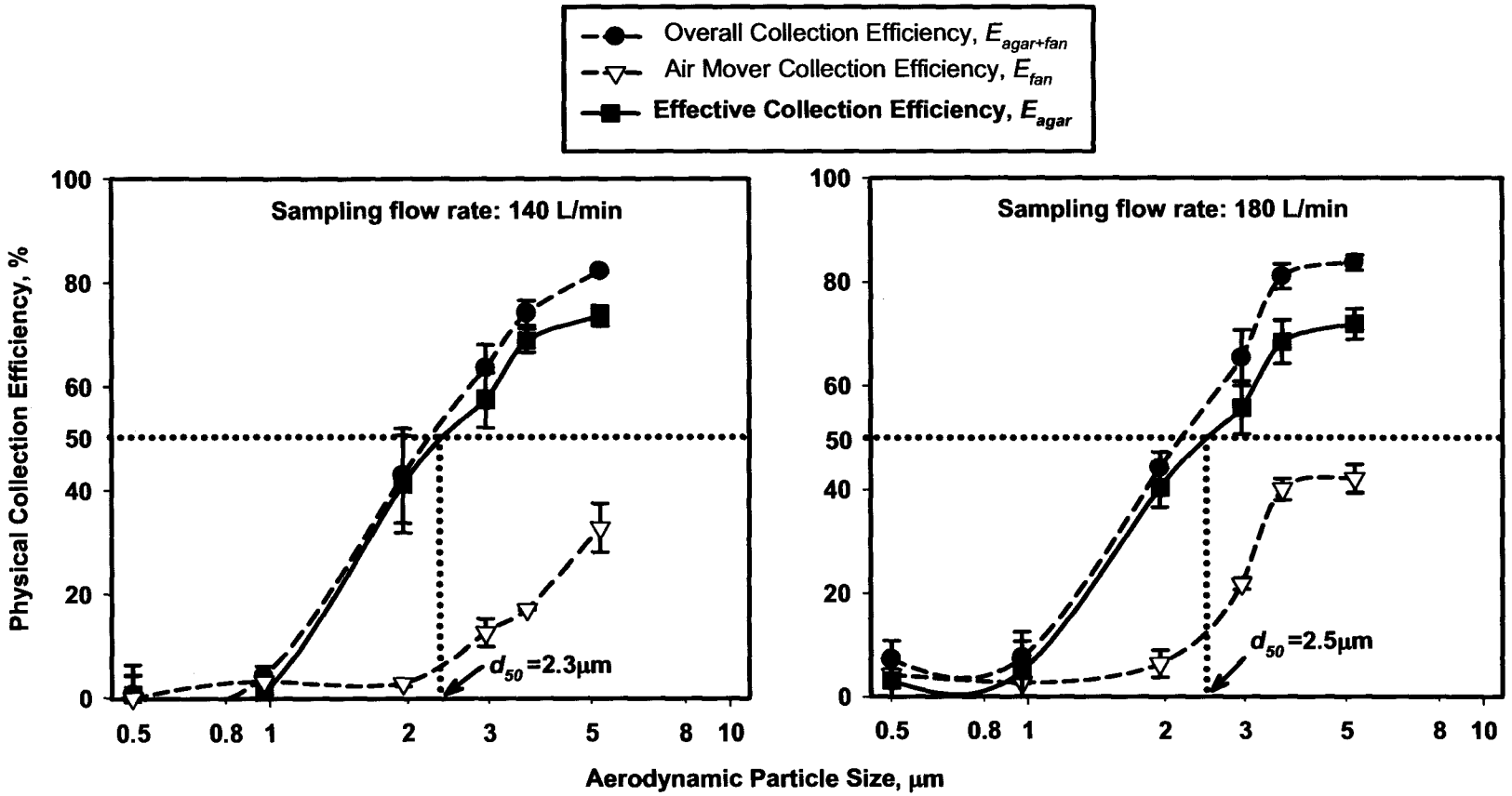

Figure 5.6. Physical collection efficiencies of Millipore Air Tester at the different sampling flow rates. Data represent averages of three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

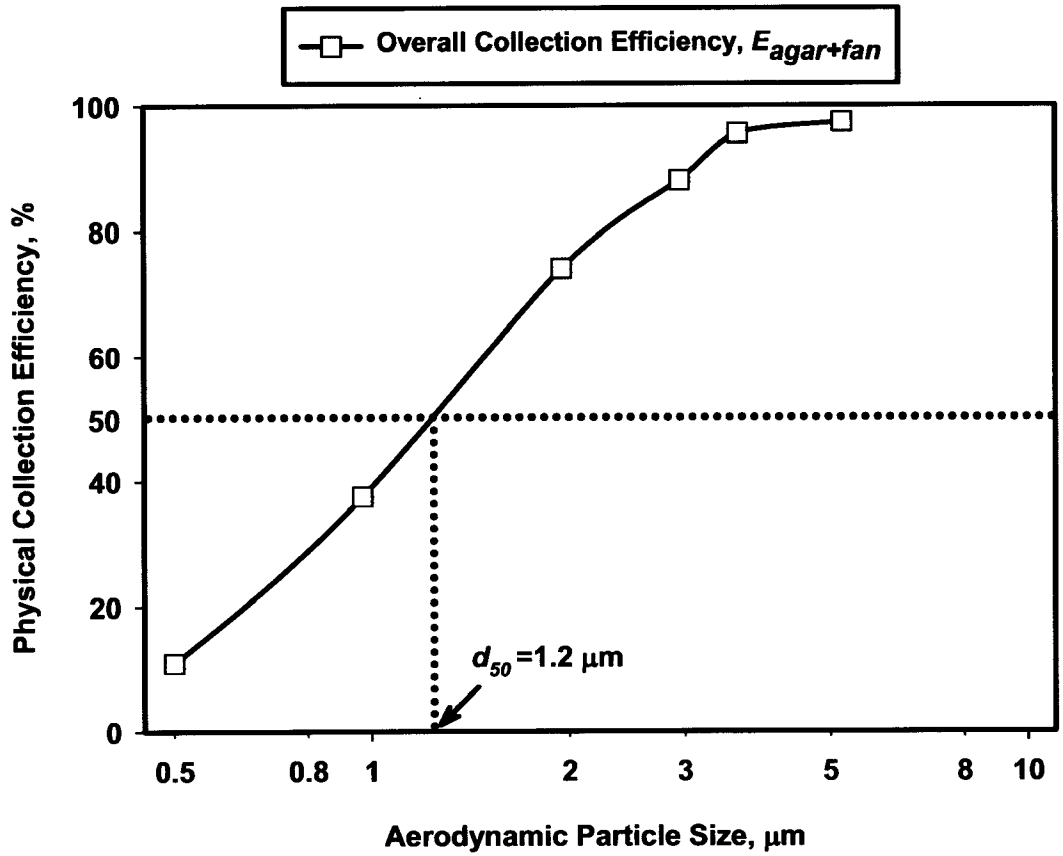

Figure 5.7. Physical collection efficiency of RCS High Flow microbial sampler. Data represent averages from three repeats and error bars stand for 1 standard deviation.

REF: Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

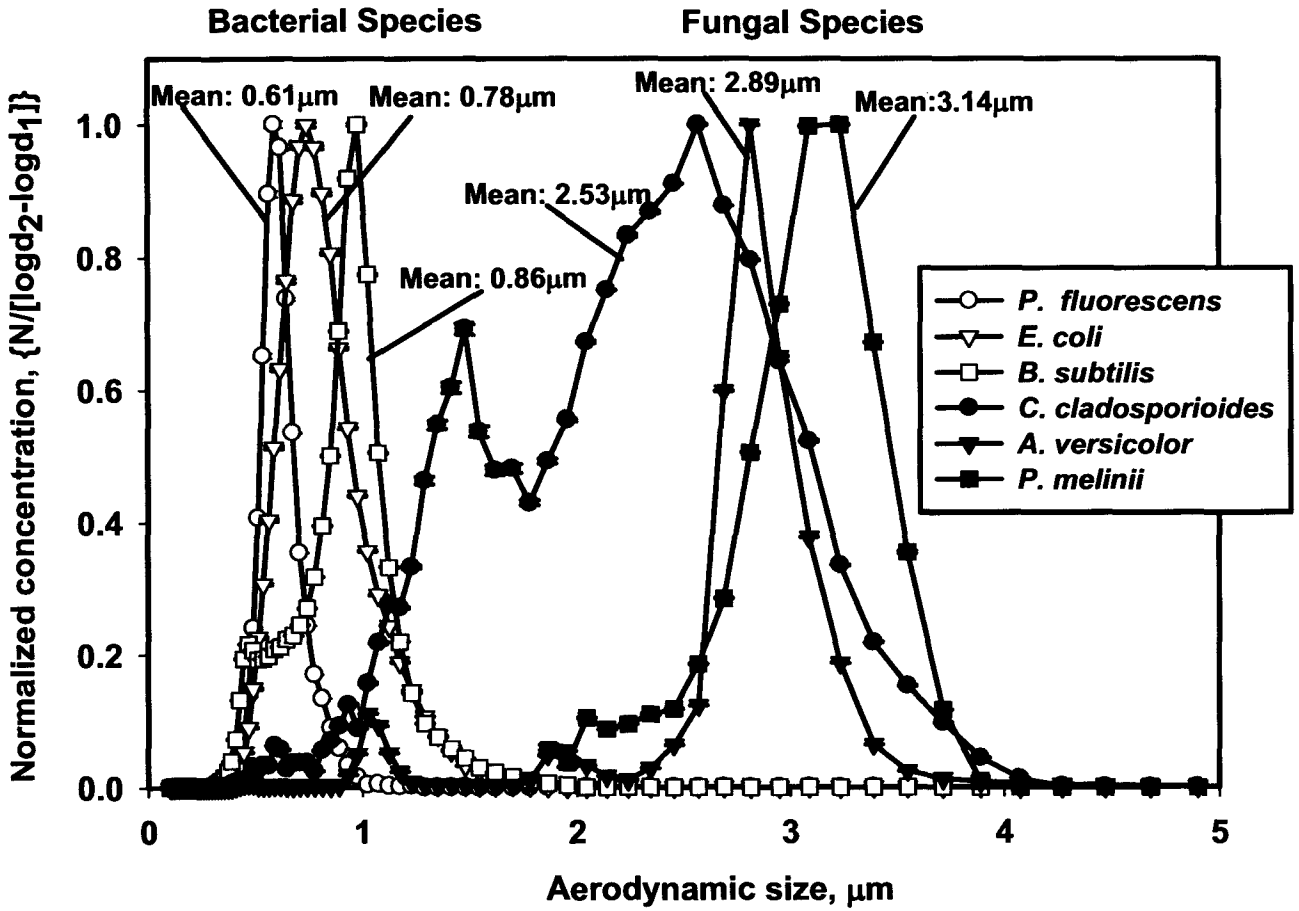

Figure 5.8. Size distribution of six microorganisms used in this study. The concentration was normalized using size giving maximum concentration.

REF: Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

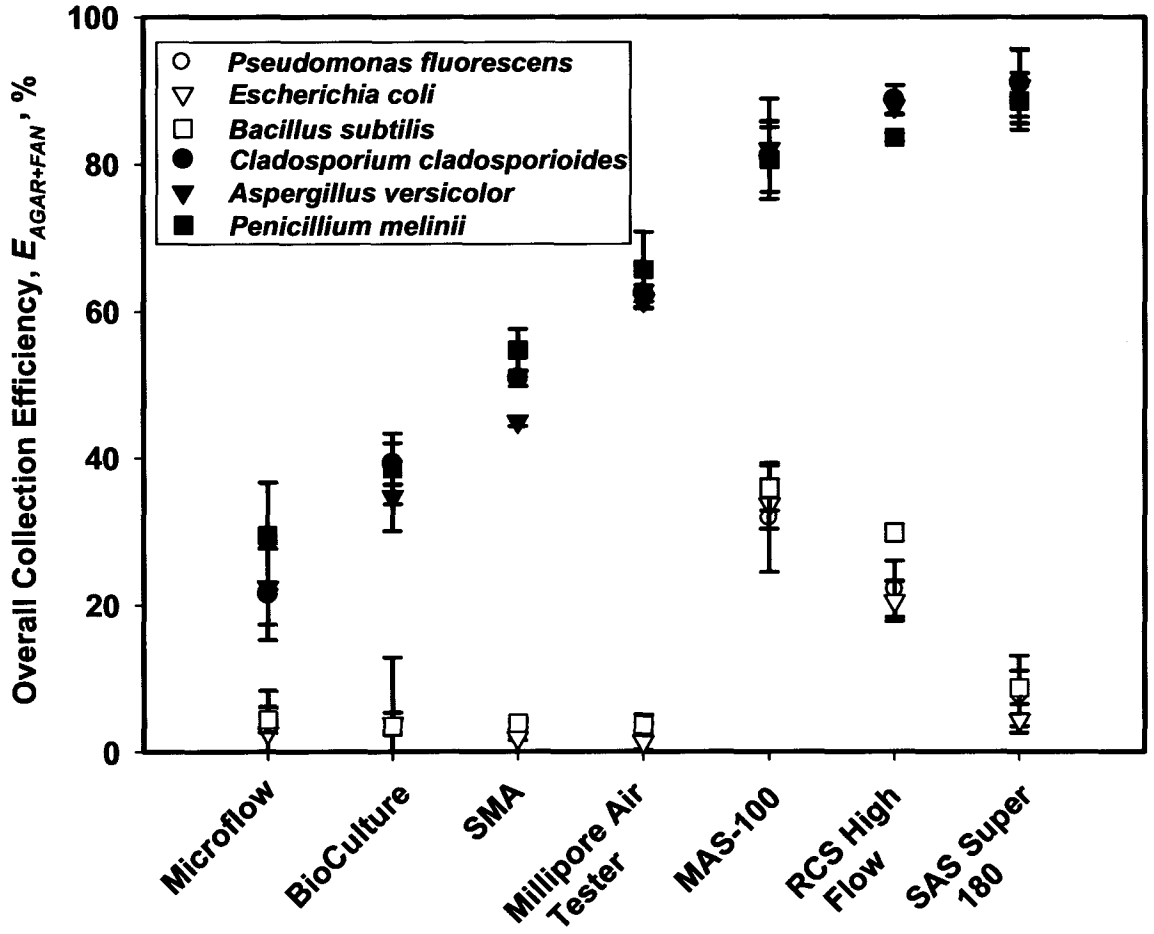

Figure 5.9. Overall physical collection efficiencies of seven portable microbial samplers when sampling bacteria and fungi.

REF: Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

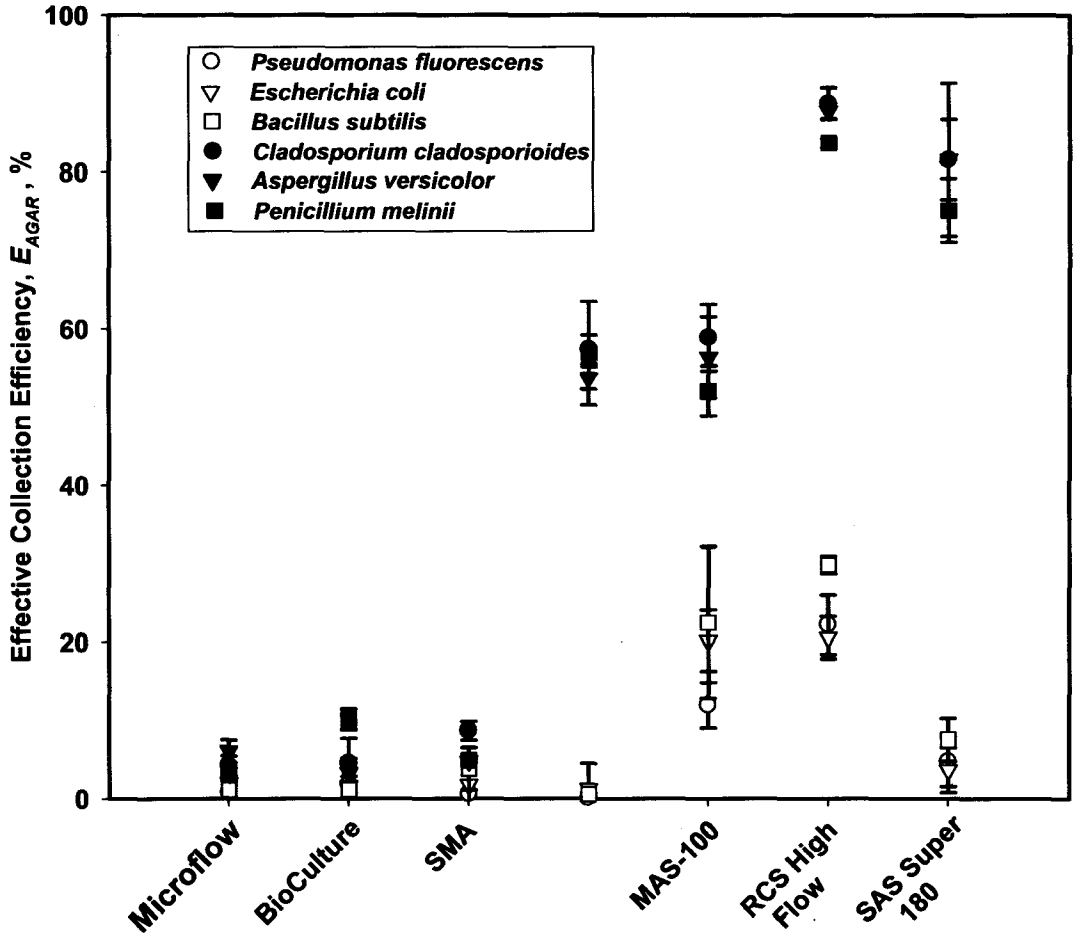

Figure 5.10. Effective collection efficiencies of seven portable microbial samplers when sampling bacteria and fungi.

REF: Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

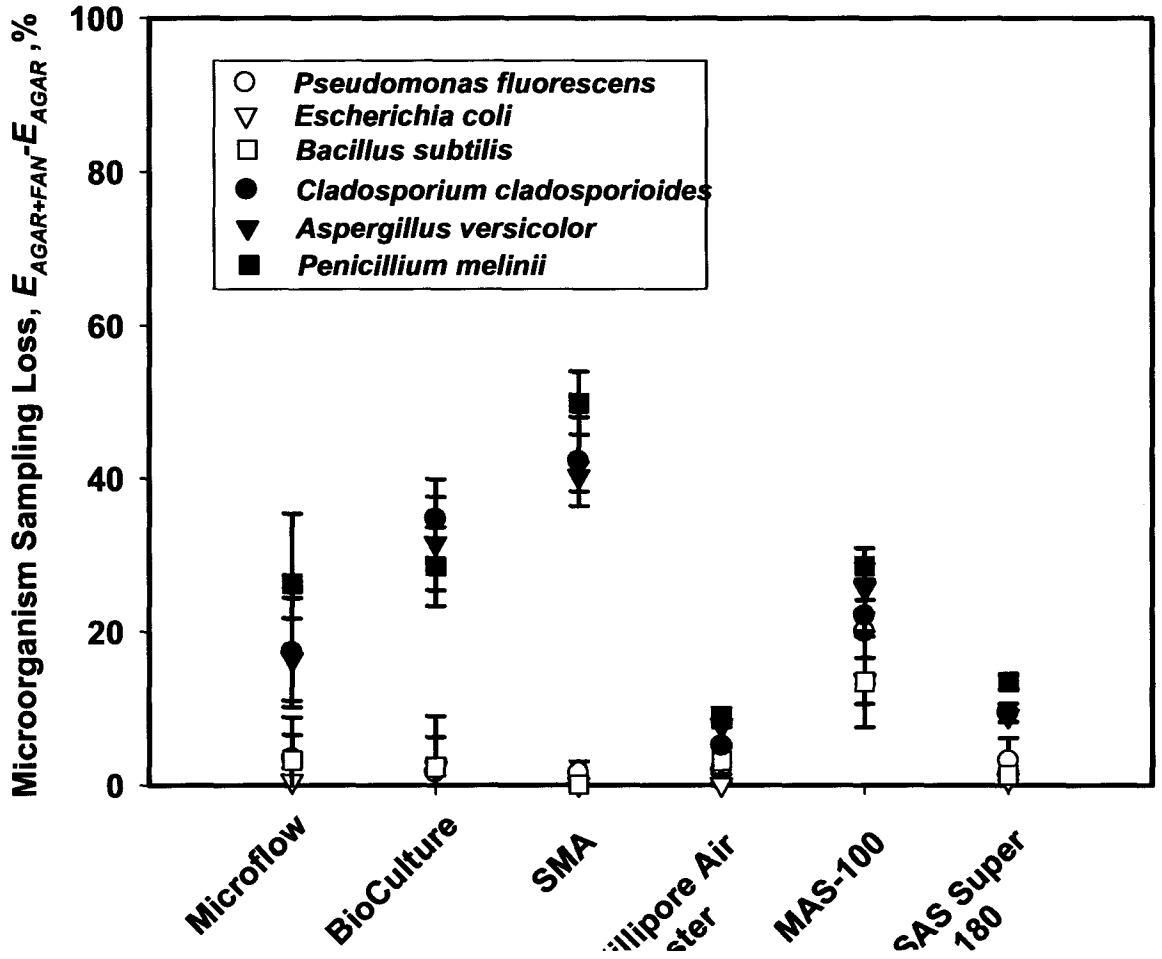

Figure 5.11. Sampling loss of six portable microbial samplers when sampling bacteria and fungi.

REF: Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

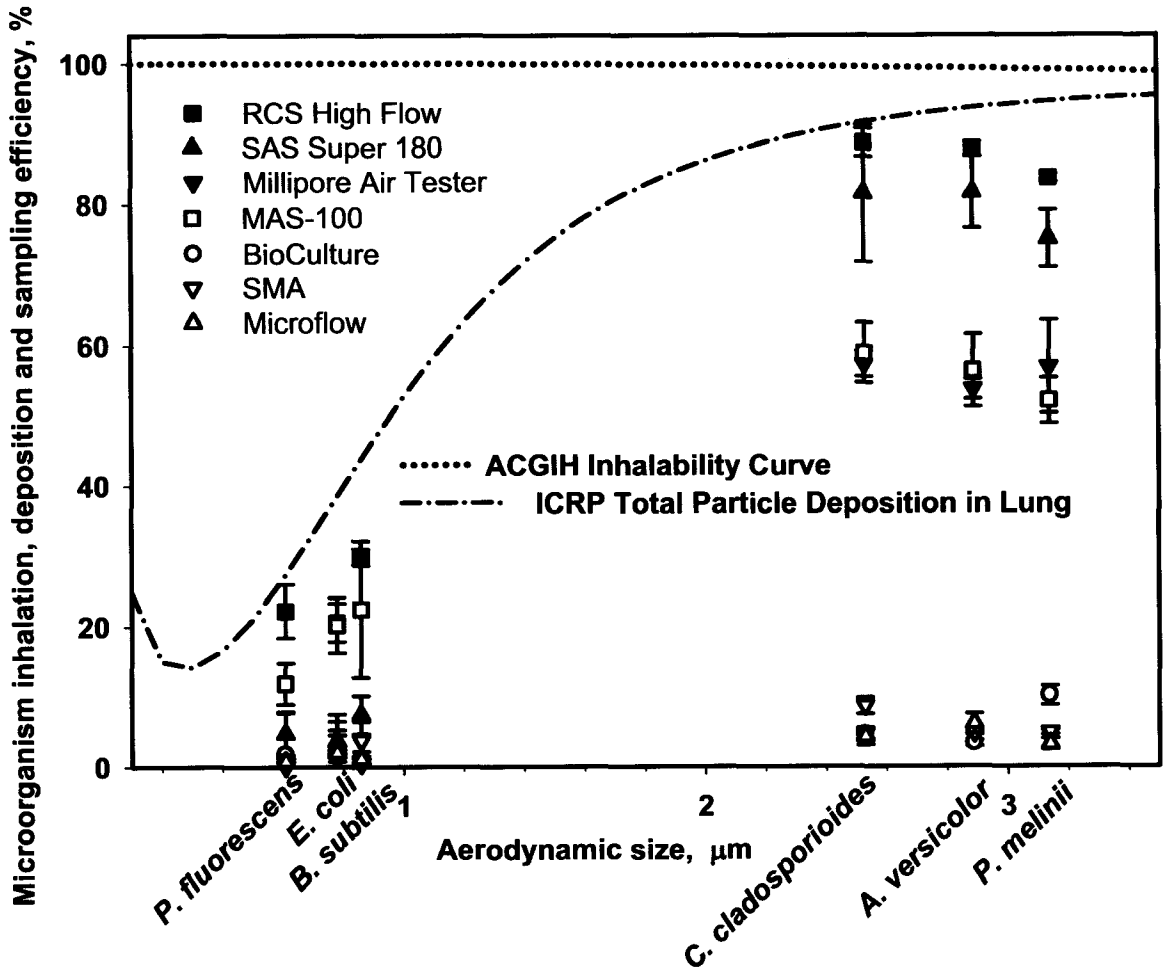

Figure 5.12. Collection performance of seven portable microbial samplers compared with total particle inhalation and deposition in the lung.

REF: Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

6 Specific Aims IV and V: *Evaluation of the biological performance of the portable bioaerosol samplers and of the inhalable reference sampler for viable bioaerosols*

In addition to sampler's ability to collect microorganisms of interest, the overall performance of any culturable bioaerosol collector also depends on its ability to maintain microorganism culturability during and after the collection. Impaction velocity was shown to affect the culturability of collected *Pseudomonas fluorescens* bacteria (Stewart et al., 1995). The biological recovery of *Escherichia coli* collected by filtration was observed to be very low compared with *Bacillus subtilis* as a result of the high desiccation stress (Li et al., 1999). These studies indicate that the extent of resistance to mechanical and desiccation stress varies among the microorganism species. In addition, the degree of microorganism embedding into the agar collection medium during the sampling might also affect their culturability (Stewart et al., 1995; Reponen et al., 1998). Thus, this part of the study was designed to investigate the effects of microorganism size, sensitivity as well as impactor parameters on microorganism recovery by portable impactors. The inhalable reference sampler (Button Sampler) equipped with gelatin filter was also included to determine its efficiency to enumerate viable organisms. The analysis of such parameters should help better understand the collection and enumeration process, thus leading to more accurate exposure assessment and improved design of future microbial samplers.

6.1 Microorganism species

The microorganism species used here as well as their preparation methods were the same as in the investigation described above.

6.2 Experimental methods

The experimental set up used in this part of the study was the same as shown in Figure 3.1 and the operation of the test set-up was described in earlier chapter. To minimize the influence of humidity on the culturability of airborne microorganisms, the humidity level inside the test chamber was kept at 40-45% using a Air-O-Swiss humidifier (Best Vacuum Inc., Chicago, IL) and monitored by a Traceable Hygrometer (Fisher Scientific, Pittsburgh, PA) (not shown in Figure 3.1) . The airborne microorganism concentrations were monitored by an optical particle counter (model 1.108, Grimm Technologies, Inc., Douglasville, GA). Airborne microorganism concentrations were adjusted by changing the aerosolization air flow rates up to 10 L/min.

The jet velocity V, of the impactors was calculated based on their sampling flow rate Q, number of jet nozzles, N, and nozzle diameter D:

$$V = \frac{4Q}{N\pi D^2}. \qquad (6.1)$$

The number of microorganisms recovered by each portable sampler was compared to that measured by a BioStage impactor (SKC Inc., Eighty Four, PA) operated at 28.3 L/min with 50mL of agar and jet velocity of 24 m/s. This sampler follows the same design as the sixth stage of the Andersen impactor which has a cut-off size of 0.65 μm (Wüst et al., 2003) and jet-to-plate distance of 1.7 mm. The Andersen impactor has been used since the fifties (Andersen, 1958) and

it has been suggested as a reference method when investigating performance of microbial samplers (Schafer et al., 1998; Predicala et al., 2002). In addition to the BioStage impactor, we also used Button Aerosol Sampler (SKC Inc., Eighty Four, PA) equipped with gelatin filter and operated at 4 L/min as a second reference sampler. This sampler has been successfully used as a personal inhalable aerosol sampler (Aizenberg et al., 2000); while gelatin filter provides high collection efficiency and nutrient rich environment (Clark et al., 2005). Our measurements indicated that Button Aerosol Sampler with gelatin filter had a collection efficiency of approximately 100% for all test microorganisms.

Before the experiments, agar plates were loaded into the portable microbial samplers and BioStage impactor, and gelatin filter along with filter support was loaded into the Button Aerosol Sampler. Trypticase soy agar plate was used for collection of bacteria and sabouraud dextrose agar plate was used to collect fungi.

To avoid overloading the sampling plates with microbial colonies (most of the samplers had 400 impaction nozzles or fewer) and to obtain the number of colony forming units (CFUs) below the upper detection limit, SAS Super 180, MAS-100, and RCS High Flow were limited to 12 sec sampling; Microflow, SMA MicroPortable, BioCulture, and Millipore Air Tester were limited to 30 sec sampling. Reference samplers BioStage impactor and Button Aerosol Sampler with gelatin filter were operated for 30 sec and 5 min, respectively. We used 5 min sampling time for the gelatin filter so that enough microorganisms would be collected at its sampling flow rate of 4 L/min without increasing the aerosolization rate.

During each experimental repeat, each of the test and reference samplers was placed inside the test chamber in a randomized order, and exposed to the same microorganism concentration for a predetermined amount of time. Five independent repeats were performed for each test microorganism with fresh bacterial or fungal suspension used in the Collison nebulizer for each repeat. Use of fresh microbial suspensions decreased the variation in microbial viability between the repeats due to aerosolization stress.

After each repeat, the agar plates were incubated directly, while the gelatine filter was dissolved in 2mL autoclaved water, and 200μL were plated onto agar plates in triplicates. After incubation, the concentrations of culturable microorganisms were determined by applying statistical correction factors to the number of Colony Forming Units (CFU) counted.

The culturable microorganism concentration, $C_{SAMPLER}$, measured either by a test sampler or by a reference sampler, was calculated using the following equation:

$$C_{SAMPLER} = \frac{N_{CFU}}{V_{AIR}}, \tag{6.2}$$

where N_{CFU} is the statistically corrected number of CFUs on an agar plate and V_{AIR} is the volume of air sampled by each sampler. For a test sampler, $C_{SAMPLER}$, can be denoted as C_{TEST}, and for BioStage and Button samplers it can be denoted as $C_{BIOSTAGE}$ and C_{BUTTON}, respectively. The relative overall efficiency, R_{EFF}, of each test sampler was calculated as the ratio of C_{TEST} versus $C_{BIOSTAGE}$; to standardize our data, performance of the Button sampler with gelatin filter was also evaluated relative to that of BioStage:

$$R_{EFF} = \frac{C_{TEST}}{C_{BIOSTAGE}}; \qquad R_{EFF} = \frac{C_{BUTTON}}{C_{BIOSTAGE}}. \tag{6.3}$$

The relative overall efficiency, R_{EFF}, i.e., the ability of a sampler to recover culturable bioaerosols relative to that of BioStage, was calculated for each repeat for all samplers, and the average values and standard deviations of R_{EFF} was calculated based on five independent repeats.

When bioaerosol samplers are exposed to the same airborne microorganism concentration, their relative performance could be governed by their effective collection efficiency, E_{COLL} (it is the same variable as E_{agar} mentioned in earlier chapters), and a stress factor, F_{STRESS}. The E_{COLL} indicates the fraction of airborne microorganisms deposited on a sampler's collection medium, while the F_{STRESS} indicates the damage caused to the microorganisms during or post-collection. Thus, the R_{EFF} in our tests could also be described by the equation below:

$$R_{EFF} = \frac{E_{COLL\_TEST}}{E_{COLL\_BIOSTAGE}} / \frac{F_{STRESS\_TEST}}{F_{STRESS\_BIOSTAGE}} = R_{COLL} / R_{STRESS}. \tag{6.4}$$

The E_{COLL} depends on a sampler's design and the microorganism size, while the F_{STRESS} is a function of microorganism sensitivity and sampler's design parameters. If the stress factors of two samplers when collecting a particular microorganism are the same ($R_{STRESS} = 1$) then the relative overall efficiency, R_{EFF}, would solely depend on the samplers' physical collection efficiency, i.e., $R_{EFF} = R_{COLL}$. Deviation from this function would indicate that the stress factors do play a role in the overall relative efficiency of the samplers, i.e., $R_{STRESS} \neq 1$.

The E_{COLL} of the tested portable impactors when collecting all six test microorganisms has already been determined earlier (Figure 5.10). The physical collection efficiencies of BioStage impactor for the microorganisms tested were estimated using the collection efficiency curve determined by other researchers (Dunbar and Mitchell, 2005) and aerodynamic microorganism diameters shown in Figure 5.8. The collection efficiency of BioStage is approximately 50% for *P. fluorescens*, 85% for *E. coli*, 90% for *B. subtilis*, and close 100% for all three fungi species.

The data analysis presented below focuses on the R_{EFF} as a function of microorganism sensitivity and stress-affecting factors of samplers' design, such as jet velocity and jet-to-plate distance.

6.3 Statistical Analysis

Single factor ANOVA available from Microsoft Excel was performed to analyze the relative overall efficiency, R_{EFF}, as a function of sampler model for a given microorganism species. Bonferoni multiple comparison test was used to make pair-wise comparisons between the samplers. Bonferoni test was chosen because it adjusts the observed significance level for the fact that multiple comparisons are made. In addition, for each test microorganism we performed linear regression analysis of R_{EFF} (dependent variable) as a function of three predictors (independent variables): physical collection efficiency, jet-to-plate distance and jet velocity. The linear regression analysis was performed for each predictor variable separately as well as performing multiple linear regression tests with all three predictors included. The different regression line fits for each microorganism were compared using adjusted R^2 statistic which takes into account the number of predictors (independent variables). When performing single and

multiple linear regression analysis we used the mean collection efficiency values which were determined in earlier. The p-values less than 0.05 indicated statistical significance in all tests.

6.4 Results when sampling bacterial species

The relative overall efficiencies of the portable samplers when collecting the sensitive and smaller (0.6 μm in aerodynamic diameter, d_{ae}) microorganism *Pseudomonas fluorescens* ranged from 0 to 0.26 as shown in Figure 6.1. Among all the samplers, the reference BioStage impactor had the highest relative overall efficiency, while none of the *P. fluorescens* bacteria collected by the Button Aerosol Sampler with gelatine filter were found culturable, most likely due to severe desiccation stress. Although gelatine filter provides a more favourable environment to collected microorganisms compared to other (non-gelatine) filters, the desiccation stress apparently was still too high for *P. fluorescens* to survive.

The SAS Super 180, MAS-100, RCS High Flow had the highest E_{COLL} for the *P. fluorescens* among the test samplers: 5, 12 and 22%, respectively. The same samplers also had the highest average relative overall efficiencies: 0.16, 0.26, and 0.24 for SAS Super 180, MAS-100, and RCS High Flow, respectively. Microflow, SMA, Millipore Air Tester, and BioCulture had average R_{EFF} values of less than 0.07, which corresponded to their $E_{COLL} < 2\%$. The results of ANOVA analysis with Bonferoni multiple pair-wise comparisons indicated that the differences among the relative efficiencies of portable samplers were not statistically significant ($p=0.06$). When R_{EFF} values for *P. fluorescens* were analyzed as a function of all impactors (including the BioStage), the difference was statistically significant ($p<0.0001$).

The regression analysis of R_{EFF} as a function of different predictor variables is presented in Table 6.1. Overall, the relative recovery of *P. fluorescens* by the tested impactors seemed to be largely influenced by the impactors' ability to physically collect this 0.6μm microorganism. i.e., the R_{EFF} had a strong linear correlation with impactors' effective collection efficiencies ($R^2=0.84$, $p<0.0001$), as shown in Figure 6.1A. The slope of the linear regression line did not statistically differ from the $R_{EFF} = R_{COLL}$ line ($p>0.05$) indicating that the stress factors between the test impactors and the BioStage were approximately equal. On the other hand, zero recovery of *P. fluorescens* from gelatine filter, which has collection efficiency of ~100%, indicates that collection efficiency is not necessarily the only factor determining the performance of a sampler and that stress factors, such as desiccation, are also important. For impactors, jet velocity was indicated as one of the parameters affecting the microbial recovery (Stewart et al., 1995). In addition, we hypothesized that jet-to-plate distance also plays a role, because it influences the dissipation of the impaction jets and thus may affect the imbedding and desiccation of the collected microorganisms. The R_{EFF} of the impactors when collecting *P. fluorescens* as a function of jet velocity and jet-to-plate distance is shown in Figure 6.1B. Individual regression analysis of R_{EFF} with jet velocity and jet-to-plate distance as predictor variables resulted in adjusted $R^2 = 0.59$ ($p<0.0001$) and adjusted $R^2 = 0.18$ ($p = 0.009$), respectively, as shown in Table 6.1. However, when all three predictor variables were included in a multiple regression model, only the collection efficiency showed a statistically significant prediction value for R_{EFF}, as shown in Table 6.1 and the R^2 virtually did not change compared to the single linear regression with collection efficiency (adjusted $R^2=0.85$, $p<0.0001$). We believe that stress-sensitive *P. fluorescens* bacteria suffer so much mechanical and desiccation stress during

collection that only the least-sensitive microorganism subpopulation survives and effect of the stress parameters of different samplers becomes obscured. The R_{EFF} data for RCS High Flow was lower than would be expected based on the collection efficiency ($p = 0.0075$) indicating additional stress induced by this centrifugal sampler.

The results obtained with *E. coli* ($d_{ae} = 0.78$ μm) were similar to those observed with *P. fluorescens*: BioStage impactor had the highest collection efficiency and the highest overall efficiency; and none of the bacteria recovered from gelatine filter were found culturable as shown in Figure 6.2A. Among other samplers, the highest relative overall efficiency, R_{EFF}, of 0.13 was observed for MAS-100, while other impactors had relative overall efficiencies ranging from 0.01 for SMA, 0.029 for BioCulture, 0.03 for Millipore and BioCulture, 0.04 for SAS to 0.07 for RCS High Flow. Single factor ANOVA with multiple comparisons indicated that all portable impactors were different from the BioStage ($p<0.0001$) and that MAS-100 was different from SMA. All other pairwise comparisons were not statistically different ($p>0.05$). Comparison of Figures 6.1B and 6.2B indicates that R_{EFF} of portable impactors when collecting *E. coli* was lower compared to sampling of *P. fluorescens*. This is likely due to the fact that BioStage has higher collection efficiency for this organism compared to *P. fluorescens* while the collection efficiencies of portable impactors when collecting *P. fluorescens* and *E. coli* are very similar, except for MAS-100. Similar to the tests with *P. fluorescens*, linear regression of *E. coli* data indicated a strong correlation between the R_{EFF} and the effective collection efficiency of the sampler (adjusted $R^2=0.94$, $p<0.0001$) and the linear regression line did not deviate from the $R_{EFF} = R_{COLL}$ line. Also, due to high susceptibility of this organism to stress, as indicated by gelatine filter data, the jet velocity and jet-to-plate distance did not seem to be the major factors affecting the overall efficiency of the impactors when collecting *E. coli* (Figure 6.2B). Multiple linear regression analysis of the R_{EFF} with all three predictor variables included did not increase the adjusted R^2 value for the model and only the collection efficiency showed statistically significant association with R_{EFF} as presented in Table 6.1. Similar to the *P. fluorescens* data, the R_{EFF} value for RCS High Flow was lower than what would be expected based on the collection efficiency of the sampler ($p<0.0001$) thus indicating that an additional stress might have been induced by this centrifugal sampler compared with other impactors.

When sampling vegetative cells of *B. subtilis* ($d_{ae} = 0.86$μm) by gelatine filter the relative overall efficiency was not different from 1.0 ($p = 0.78$) as shown in Figure 6.3A. This indicates that the cells were equally susceptible to collection stress induced by the BioStage impactor and gelatin filter. MAS-100 and RCS High Flow had R_{EFF} values of approximately 0.6, while SAS had a value of 0.5 as seen in Figure 6.3A. Millipore, Microflow, SMA, and BioCulture had relative overall efficiency rates of less than 0.2. The results from single factor ANOVA analysis with multiple pair-wise comparisons indicated that all portable samplers were statistically different from the BioStage ($p<0.0001$), while the relative biological recovery rates of RCS High Flow, MAS-100, and SAS were not statistically different. The R_{EFF} values by BioCulture, Microflow, Millipore and SMA were also not statistically different. Any pairwise comparison between the individual samplers from the two groups was statistically significant. Linear regression of R_{EFF} impactor values with collection efficiencies showed adjusted R^2 value of 0.74, which was lower than that observed for *E. coli* and *P. fluorescens*. The SAS Super 180 and Millipore Air Tester had R_{EFF} values statistically above the $R_{EFF} = R_{COLL}$ line and the intercept of the linear regression line was above zero value ($p = 0.002$). This suggested that the high jet velocity of BioStage (24

m/s) might be causing the damage to *B. subtilis* vegetative cells compared to portable impactors which have lower jet velocities (Figure 6.3B). In addition, portable samplers have larger jet-to-plate distances compared to the BioStage (Figure 6.3B) causing the jets to dissipate thus providing more optimum embedding conditions. However, inclusion of all three predictors (collection efficiency, jet-to-plate distance and jet velocity) in the multiple regression model did not improve the regression line fit (adjusted $R^2 = 0.75$) and among the tree predictors only the collection efficiency was statistically significant ($p = 0.02$) as shown in Table 6.1. The intercept value was also not statistically different from zero ($p = 0.05$).

When sampling bacterial species, the relative overall performance of the samplers largely depended on their physical collection efficiencies, and the inclusion of the jet-to-plate distance and jet velocity as predictors in a multiple linear regression model did not show their statistical significance as presented in Table 6.1. In addition, none of the *P. fluorescens* and *E. coli* bacteria recovered when collected on the gelatin filter, likely due to desiccation stress.

6.5 Results when sampling fungal species

The results when sampling fungal spores of *Cladosporium cladosporioides* ($d_{ae} = 2.53$ μm) are presented in Figure 6.4. The mean relative overall efficiency when sampling with gelatin filter was 0.17 and was statistically lower than unity ($p<0.0001$), which indicated that *C. cladosporioides* is more sensitive to stress induced by sampling onto gelatin filter than to stress when collected by the BioStage. MAS-100, SAS Super 180 and RCS High Flow samplers had mean R_{EFF} values of 1.08, 0.97, and 0.74, respectively, much higher than other samplers. Compared to the expected performance based on the $R_{EFF} = R_{COLL}$ curve, Microflow, BioCulture, MAS-100 and SAS Super 180 samplers performed better, while SMA and Millipore Air Tester performed worse ($p <0.05$) as shown in Figure 6.4A. The average relative efficiency of RCS High Flow sampler was not statistically different from the value predicted by the $R_{EFF} = R_{COLL}$ curve ($p=0.07$). The results of multiple pair-wise comparisons indicated that the R_{EFF} of MAS-100 and SAS Super 180 were not statistically different from that of the BioStage impactor, although they have lower effective collection efficiencies. These results suggested that when sampling *C. cladosporioides*, not only a sampler's collection efficiency, but also other parameters might influence its overall performance. In addition, the RCS was not different from the SAS Super 180 and there were no differences among BioCulture – Microflow – Millipore and Microflow-SMA. Any other pair-wise comparisons were statistically different.

The linear regression of R_{EFF} as a function of the collection efficiency alone yielded adjusted $R^2 = 0.65$ (Table 6.1) which was lower than that observed for bacteria. In addition, the intercept value was statistically different from zero ($p = 0.04$). When jet-to-plate distance and jet velocity were separately used as single predictors for the R_{EFF}, the linear regressions yielded adjusted R^2 values of 0.13 and 0.42, respectively ($p<0.0001$). When all three predictors were included in the multiple linear regression model, the adjusted R^2 increased to 0.75 ($p<0.0001$) and the effects of collection efficiency and jet-to-plate distance was statistically significant, while the effect of jet velocity was not statistically significant as shown in Table 6.1.

The statistical significance of jet-to-plate distance could be related to the embedding and desiccation stress of microorganisms. It has been indicated that the degree of embedding of collected microorganisms by impactors may affect their biological recovery. Insufficient

embedding may limit the microorganism's access to the moisture and nutrients, while sustaining additional dehydration during the sampling (Willeke et al., 1995). On the other hand, excess embedding of microorganisms such as actinomycete species *Streptomyces albus* was observed to completely inhibit their growth - no colonies were observed (Reponen et al., 1998). In addition, a sampler's large jet-to-plate distance decreases its collection efficiency, likely due to dissipation of impaction jets as was indicated in Chapter 5. Dissipated, or wider, jets decrease the embedding and also desiccate the agar not only in the immediate impaction areas, but also at the neighbouring regions thus causing desiccation stress to the collected organisms. As indicated by the gelatin filter results, the *C. cladosporioides* is rather susceptible to the desiccation stress.

A high impaction velocity and short jet-to-plate distance of BioStage (Figure 6.4B) might have resulted in excess embedding of *C. cladosporioides* spores. The SAS Super 180 and MAS-100 have successively lower impaction velocities and larger jet-to-plate distances, which result in more optimum embedding conditions for the microorganisms while still ensuring their collection efficiencies of 82% and 60%, respectively. Millipore Air Tester has effective collection efficiency similar to that of MAS-100, but its relative overall efficiency was approximately 0.36, or about twice lower compared to MAS-100. We believe that this observation is a result of sampler's large jet-to-plate distance, which causes the impaction jets to dissipate. Jets that dissipate too much do not ensure sufficient embedding and desiccate the agar not only in the immediate impaction areas, but also at the neighbouring regions thus causing desiccation stress to the collected organisms. The same explanation could be applied to SMA, which also has relatively large jet-to-plate distance. The BioCulture and Microflow have low jet velocities but also short jet-to-plate distances (Figure 6.4B) which resulted in their relative efficiencies R_{EFF} of 0.3 and 0.18 – higher than predicted by collection efficiencies of less than 5%. The low jet velocities are responsible for low collection efficiencies of the samplers. However, those few particles that are collected suffer very minimal impaction stress. In addition, the *C. cladosporioides* have rather wide size distribution (Figure 5.8). Thus it is likely that due to low jet velocities these two samplers collect only the largest spores which could be better suited to withstand limited access to nutrients and the desiccation stress. Therefore, performance of BioCulture and Microflow was better than predicted by $R_{EFF} = R_{COLL}$ curve.

The data with *Aspergillus versicolor* (d_{ae} = 2.89 μm) shown in Figure 6.5 indicate that this microorganism's susceptibility to desiccation stress was statistically the same as its susceptibility to impaction stress of the BioStage (p = 0.91). The mean R_{EFF} value for gelatin filter was 1.2 which could be explained by a slightly lower collection efficiency of the BioStage impactor compared to the filter. Multiple pair-wise comparisons for *A. versicolor* indicated that R_{EFF} values of all portable impactors were statistically different compared to the BioStage. The RCS High Flow, SAS Super 180 and MAS-100 had relative overall efficiencies of approximately 0.5, and they were not statistically different. BioCulture, Microflow, Millipore and SMA had R_{EFF} values of less than 0.2 and multiple pair-wise comparisons indicated that they were not statistically different except for Millipore versus SMA. Other pair-wise comparisons were statistically different. The R_{EFF} value of MAS-100 did not differ statistically from that predicted by $R_{EFF} = R_{COLL}$ (p = 0.54). The performance of BioCulture was better than predicted by the collection efficiency alone (p = 0.002), while performance of other portable impactors was statistically lower than predicted by the collection efficiency alone. When collection efficiency was used as a single predictor in the linear regression model it yielded adjusted $R^2 = 0.73$

($p<0.0001$), and the slope coefficient of the regression line was statistically different from that of $R_{EFF} = E_{COLL}$ curve ($p < 0.05$). This result indicated that not only the collection efficiency, but also other factors play a role in predicting the performance of the impactors. When all three predictors were used in the multiple regression model, the adjusted R^2 increased to 0.89 ($p<0.0001$) indicating a better fit and all three predictor variables – collection efficiency, jet-to-plate distance, and jet velocity - were statistically significant as shown in Table 6.1.

We believe that for this microorganism high jet velocity and resulting impaction velocity are needed to ensure not only its collection, but also its sufficient embedding. Since all the portable impactors have lower jet velocities and larger jet-to-plate distances compared to the BioStage (Figure 6.5B), they produced less-then-optimal embedding of the *A. versicolor*. The exceptions to this explanation are performances of MAS-100 and BioCulture, which also have lower jet velocities, but nonetheless performed as predicted by the collection efficiency or better. BioCulture has a cut-off size of 7μm with the sharp inclination of the collection efficiency curve starting at 3μm (Figure 5.2). Thus, this sampler collects larger spores that might be better suited to withstand limited access to nutrients. Exclusion of the MAS-100 from the multiple regression model yielded R^2 =0.93 with all three predictor variables being significant. Thus, for the MAS-100, we speculate that a yet unaccounted design factor, such as specific arrangement of impaction nozzles (sun ray pattern), makes its overall performance better than could be predicted based on the current model. The effect of multiple nozzle arrangement on the impactor collection efficiency due to the changes in the location of the jet stagnation points was shown by other researcfhers (Kwon et al., 2002). We speculate that changes in the jet stagnation points affect the desiccation and subsequently survival of the microorganisms.

The data when sampling *Penicillium melinii* (d_{ae} = 3.14 μm) are presented in Figure 6.6. The relative overall performance of gelatine filter was not statistically different from 1 as shown in Figure 6.6A ($p = 0.96$), indicating that this specie is also a hardy microorganism sustaining impaction and desiccation stress equally well. MAS-100 had a highest R_{EFF} value of 0.78, while SAS Super 180 and RCS High Flow had R_{EFF} values of 0.48 and 0.41, respectively. The ANOVA test with multiple pair-wise comparisons indicated that the latter two samplers were not statistically different. Millipore Air Tester had a relative overall efficiency of 0.22, while Microflow, SMA, and BioCulture samplers had R_{EFF} values of less than 0.1. The performance of the latter three samplers was not statistically different. Any other pair-wise comparison, including that of portable impactors against the BioStage, was statistically different. Overall, the data profile for *P. melinii* seems to resemble that of *A. versicolor*. The MAS-100 performed better than could be predicted by the collection efficiency ($p = 0.001$), while the other samplers performed worse ($p < 0.05$). Linear regression of R_{EFF} using collection efficiency as the sole predictor variable yielded adjusted $R^2 = 0.61$ ($p<0.0001$), lowest among the investigated microorganisms. In addition, the slope coefficient of this regression line was statistically different from that of $R_{EFF} = E_{COLL}$ ($p < 0.05$). This result indicated that not only the collection efficiency, but also other factors play a role in predicting the performance of the impactors when collecting the *P. melinii*. When all three predictors were used in the multiple regression model, the adjusted R^2 increased to 0.80 ($p<0.0001$) indicating a better regression line fit. Both predictors associated with embedding - jet-to-plate distance and jet velocity - were statistically significant as shown in Table 6.1. Interestingly enough, the collection efficiency was not statistically significant in this regression model.

Again, similar to the data with *A. versicolor* we believe that the *P. melinii* requires good imbedding for its optimal growth. Compared to the BioStage, all of the tested impactors have lower jet velocities and larger jet-to-plate distances (Figure 6.6B), which result in lower impaction velocities and thus in less-than-optimal embedding of the spores. Therefore, their overall performance was worse than predicted by the $R_{EFF} = R_{COLL}$ curve. Based on the multiple regression model including all samplers, jet-to-plate distance and jet velocity (but not collection efficiency) could be used to predict the performance of the impactors when collecting *P. melinii* with adjusted $R^2 = 0.8$. The only exception to this explanation is again the MAS-100 impactor which, compared to the BioStage, has lower jet velocity and large jet-to-plate distance, but still performed better than predicted by the $R_{EFF} = R_{COLL}$ curve. In fact, exclusion of MAS-100 from the multiple regression model yielded the adjusted $R^2 = 0.95$ ($p<0.0001$) with all three predictor variables being statistically significant. Again, similar to *A. versicolor* data we believe that a yet unaccounted factor in MAS-100 design, such as specific arrangement pattern of impaction nozzles, improves its overall performance.

6.6 Discussion

When enumerating single bacterial cells, the BioStage impactor performed better than portable impactors. When enumerating single airborne fungal spores, the BioStage reference impactor also performed better than portable impactors, except for *C. cladosporioides*, when MAS-100 and SAS Super 180 performed equally well. Overall, among the investigated portable samplers, the MAS-100 impactor seemed to perform the best, while the RCS High Flow and the SAS Super 180 often had somewhat lower relative efficiencies. One has to note that this research analyzed impactor efficiency relative to the BioStage. If the BioStage has a low biological performance when sampling sensitive bacteria, then even a high relative efficiency of a portable impactor does not indicate its high absolute biological performance.

Overall, when sampling fungi, the relative efficiency of the test portable impactors, R_{EFF}, correlated worse with the impactor's collection efficiency compared to the sampling of bacteria. For fungal sampling, the adjusted R^2 ranged from 0.61 to 0.73, while for bacterial sampling it ranged from 0.74 to 0.94. However, even for fungi, the linear regression could be used to predict their performance as a first approximation. For sampling of fungi, when other parameters of the impactor design, such as jet velocity and jet-to-plate distance were included in the multiple linear regression, the adjusted R^2 increased to a range of 0.75 - 0.89. Inclusion of the same factors in the multiple linear regression analysis for bacterial sampling virtually did not affect the value of adjusted R^2. Certain other factors, as demonstrated by MAS-100 when sampling *A. versicolor* and *P. melinii,* also seem to play a performance - modifying role.

In addition, when sampling on gelatin filter we also observed zero recovery of *P. fluorescens* and *E. coli* bacteria and lower recovery of *C. cladosporioides* fungal spores compared to the BioStage. The gelatin filter was used with 5 minute sampling time so that enough microorganisms would be collected without increasing the aerosolization rate. The complete absence of bacterial colonies and lower recovery of *C. cladosporioides* after such a relatively short sampling time also indicates the susceptibility of these microorganisms to desiccation stress.

Based on the data obtained with several impactors when sampling different microorganism species, a general function of an impactor's overall performance could be described using the following function:

$$R_{BIO} = f(\alpha, \beta)$$

where R_{BIO} is the overall performance of the sampler, $\boldsymbol{\alpha}$ is the ability of the sampler to physically collect microorganisms; and $\boldsymbol{\beta}$ is the ability of the sampler to preserve the culturability of the microorganism during or after the collection.

The function $\boldsymbol{\alpha}$ largely depends on the jet velocity, jet-to-plate distance, and microorganism size, while the function $\boldsymbol{\beta}$ depends on sensitivity of microorganism species, i.e., the ability to recover from stress, jet-to-plate distance, and jet velocity. The latter two factors will determine the degree of microorganism's embedding into agar collection medium. These factors are also associated with the degree of mechanical stress imparted on the organism. In certain cases, there might exist a competing influence between the jet velocity and jet-to-plate distance on the overall performance of the sampler, R_{BIO}. Sampling time, environmental factors (humidity level and temperature) and human errors could also affect the accurate characterization of culturable microorganism concentrations by the impactor in the air. As we observed with the MAS-100 impactor when sampling fungal spores, there might also be an extra factor affecting the overall performance of the sampler.

This part of the study investigated the overall performance of portable impactors as a function of microorganism species and impactor parameters. The results are hoped to contribute towards understanding of the factors affecting microorganism enumeration by microbial impactors. The obtained data on the overall sampler performance will serve as references when selecting a portable sampler for environmental monitoring and when designing new microbial samplers for collection of culturable bioaerosols.

6.7 Tables and Figures for Chapter 6

Table 6.1. Results from single and multiple linear regression analysis. REF: Yao M and Mainelis G: [2006]. Journal of Aerosol Science 37(11): 1467-1483.

Regression Analysis	**Regression report for test microorganisms**						
Single predictor variables in a single linear regression		***Pseudomonas fluorescens***	***Escherichia coli***	***Bacillus subtilis***	***Cladosporium cladosporioides***	***Aspergillus versicolor***	***Penicillium melinii***
Collection efficiency	Adjusted R^2	0.84	0.94	0.74	0.65	0.73	0.61
	p value for variable	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Jet-to-plate distance	Adjusted R^2	0.18	0.10	0.11	0.13	0.10	0.10
	p value for variable	0.009	0.025	0.04	0.01	0.03	0.03
Jet velocity	Adjusted R^2	0.59	0.55	0.54	0.42	0.71	0.64
	p value for variable	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
All three predictor variables in a multiple linear regression							
	Adjusted R^2 for multiple regression	0.85	0.94	0.75	0.75	0.89	0.80
	p value for F statistic (probability of Type I error)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Collection efficiency	*p* value for variable	<0.0001	<0.0001	0.02	<0.0001	0.0006	0.08
Jet-to-plate distance	*p* value for variable	0.051	0.6	0.16	0.0002	<0.0001	<0.0001
Jet velocity	*p* value for variable	0.053	0.25	0.08	0.55	<0.0001	<0.0001

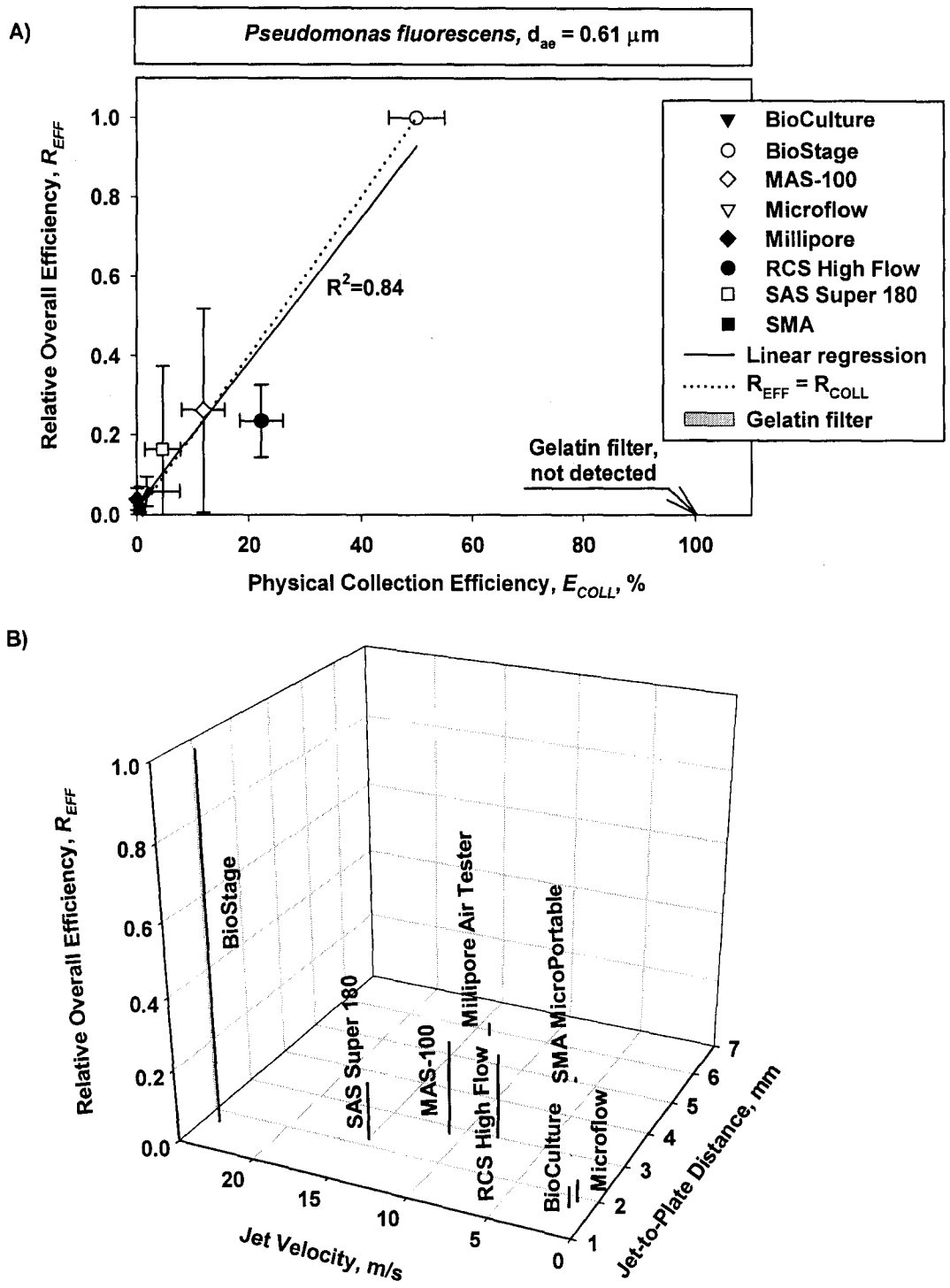

Figure 6.1. Relative overall performance of bioaerosol samplers when collecting *Pseudomonas fluorescens* bacterial cells as a function of different parameters: A) Effective collection efficiency; B) Jet velocity and jet-to-plate distance. The data represent averages from five repeats and error bars represent one standard deviation.

REF: Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

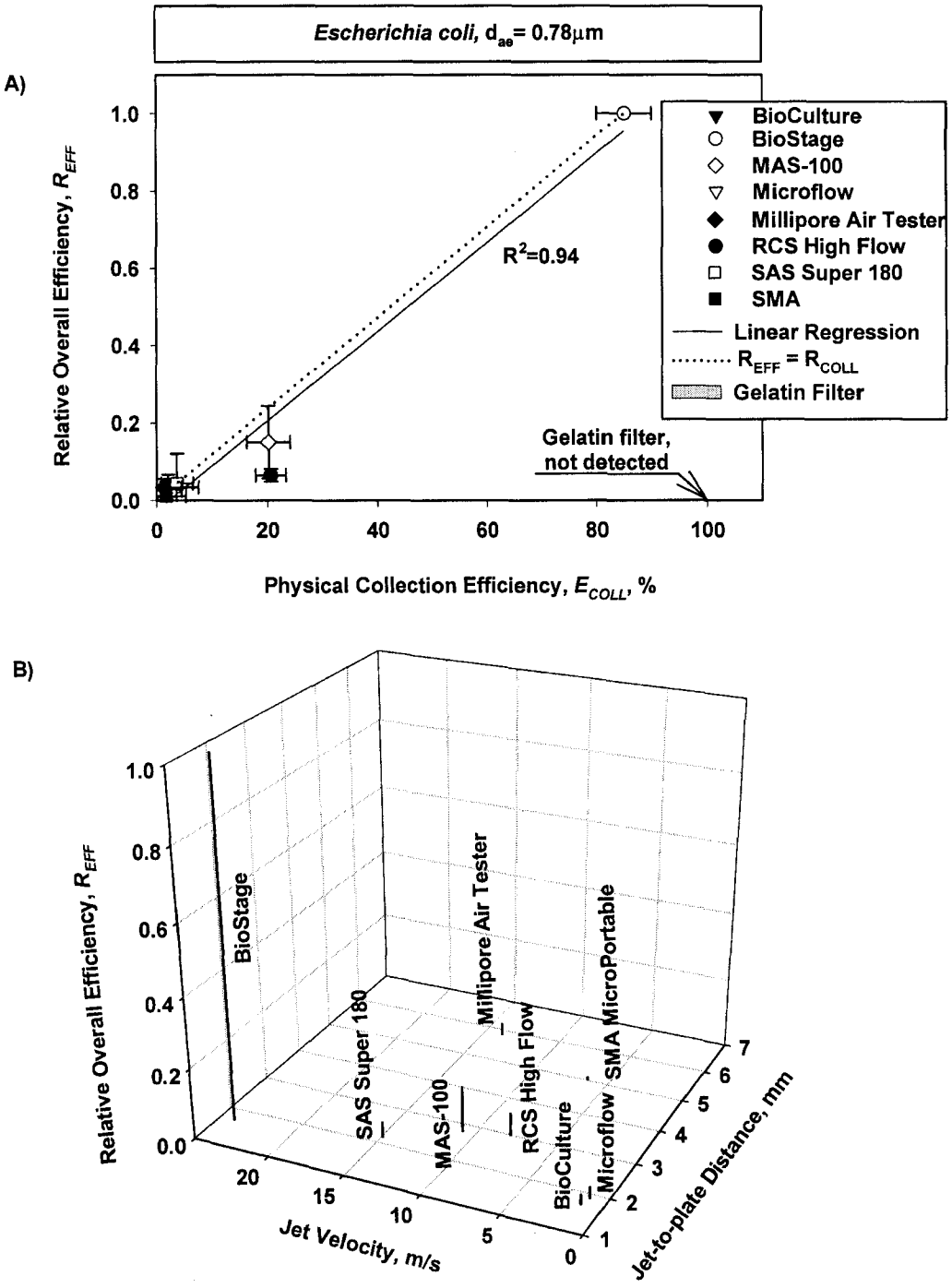

Figure 6.2. Relative overall performance of bioaerosol samplers when collecting *Escherichia coli* bacterial cells as a function of different parameters: A) Effective collection efficiency; B) Jet velocity and jet-to-plate distance. The data represent averages from five repeats and error bars represent one standard deviation.

REF: Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

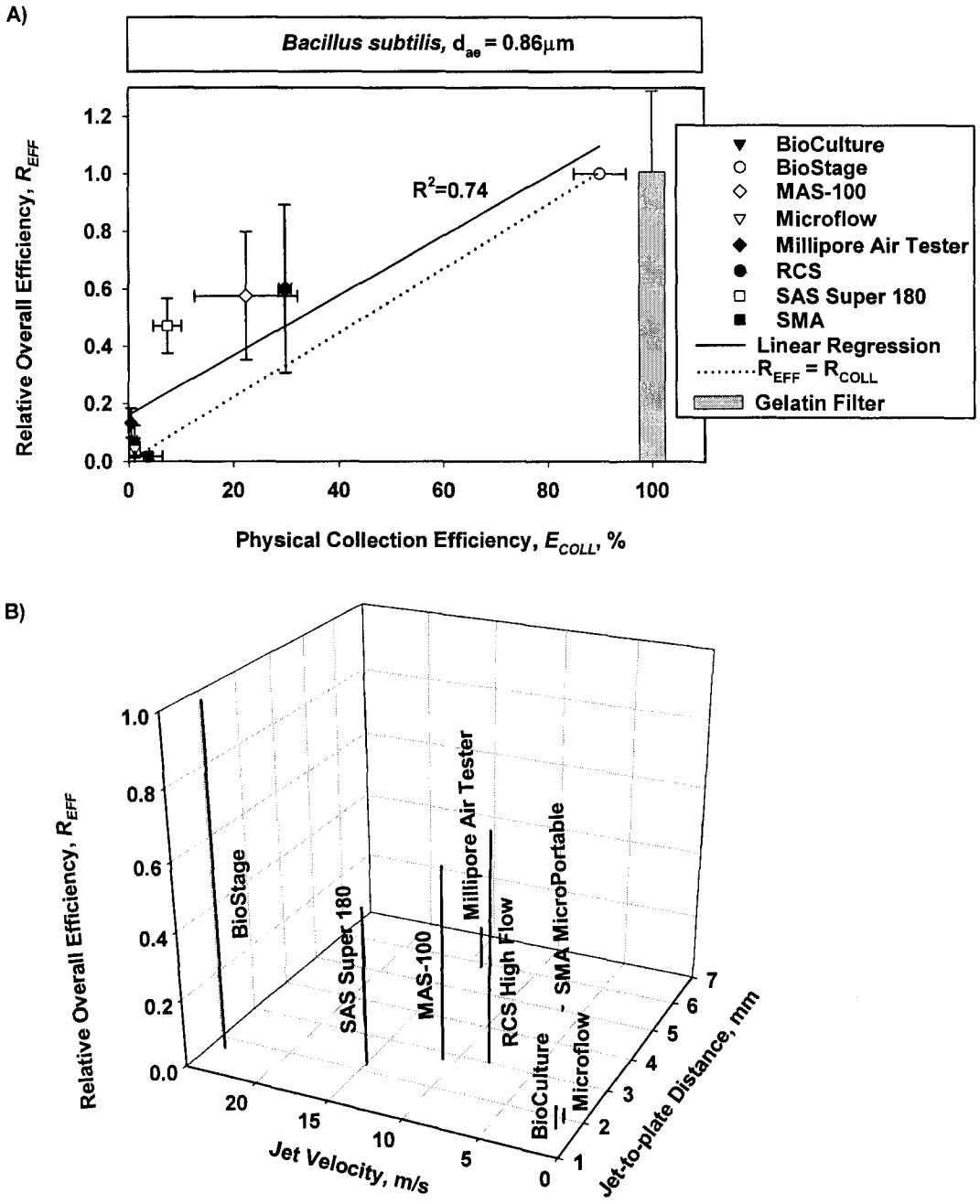

Figure 6.3. Relative overall performance of bioaerosol samplers when collecting *Bacillus subtilis* bacterial cells as a function of different parameters: A) Effective collection efficiency; B) Jet velocity and jet-to-plate distance. The data represent averages from five repeats and error bars represent one standard deviation.

REF: Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

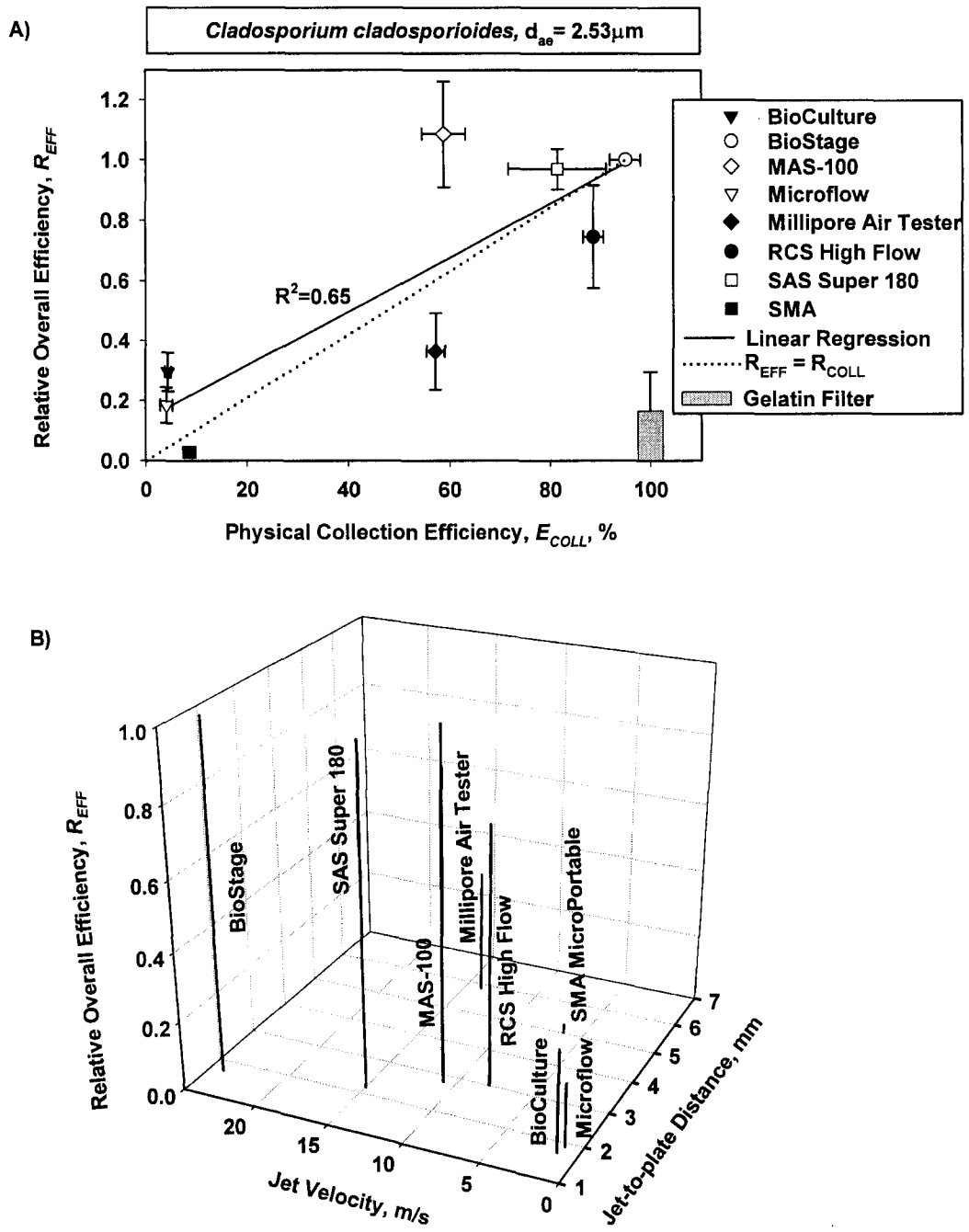

Figure 6.4. Relative overall performance of bioaerosol samplers when collecting *Cladosporium cladosporioides* fungal spores as a function of different parameters: A) Effective collection efficiency; B) Jet velocity and jet-to-plate distance. The data represent averages from five repeats and error bars represent one standard deviation.

REF: Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

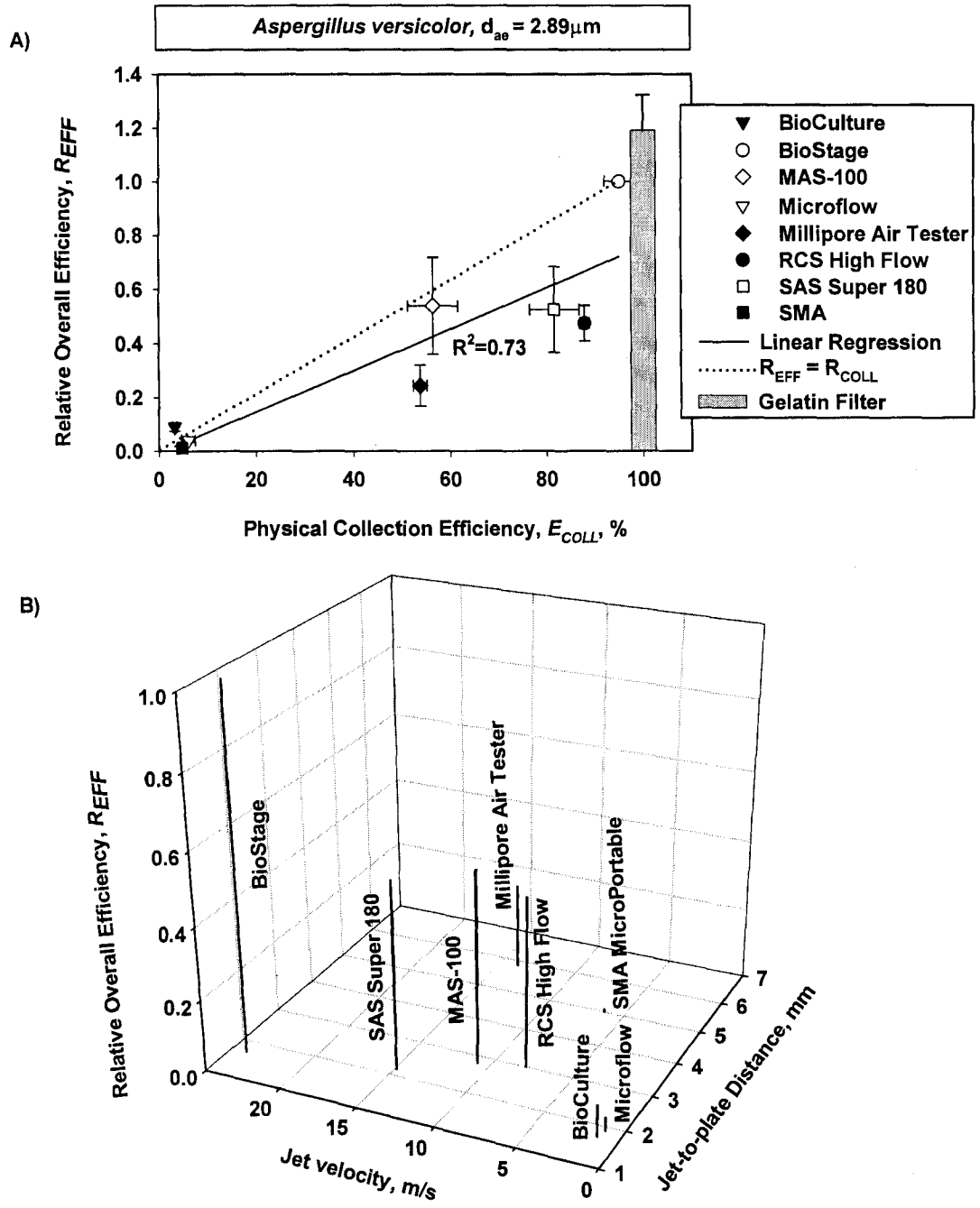

Figure 6.5. Relative overall performance of bioaerosol samplers when collecting *Aspergillus versicolor* fungal spores as a function of different parameters: A) Effective collection efficiency; B) Jet velocity and jet-to-plate distance. The data represent averages from five repeats and error bars represent one standard deviation.

REF: Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

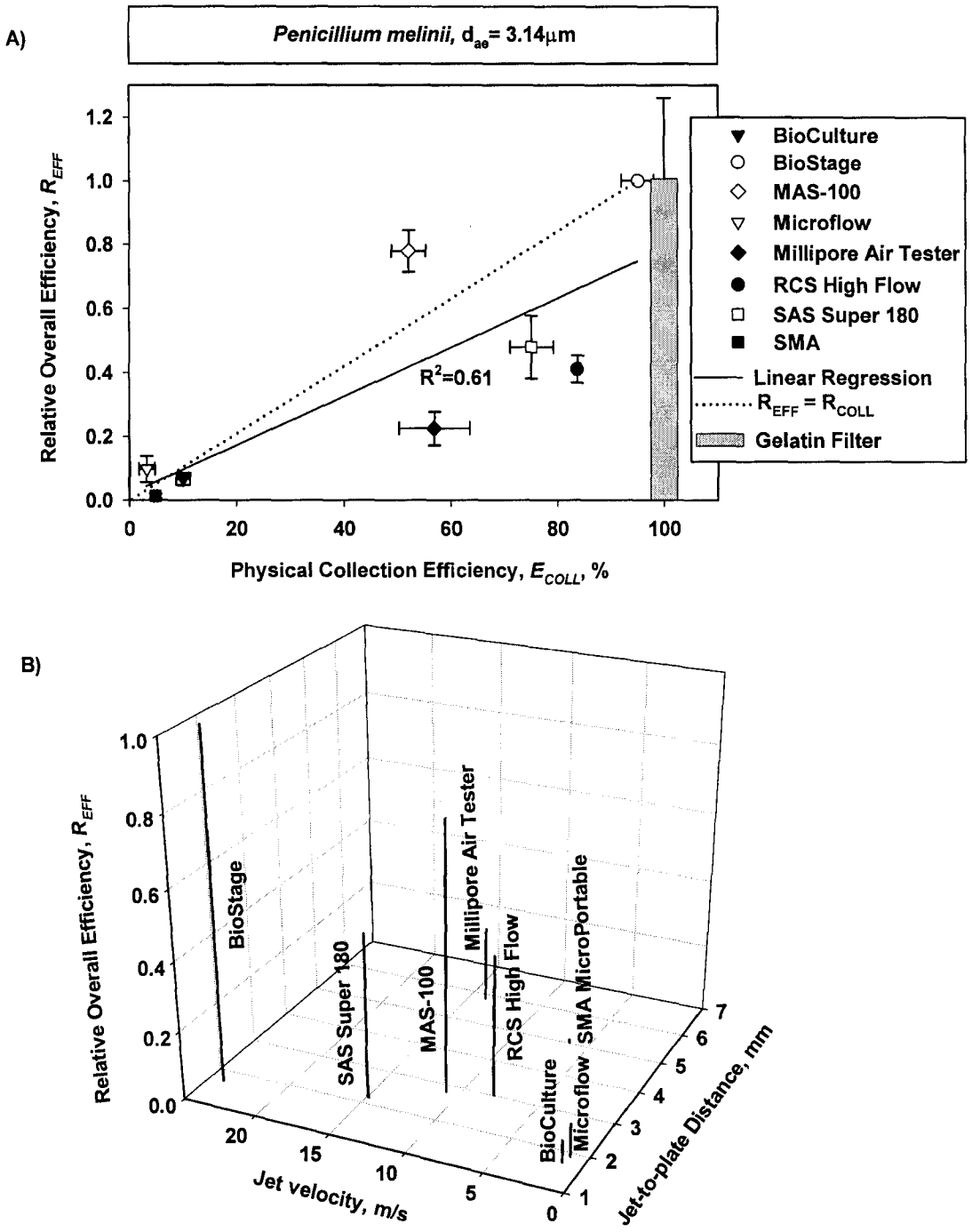

Figure 6.6. Relative overall performance of bioaerosol samplers when collecting *Penicillium melinii* fungal spores as a function of different parameters: A) Effective collection efficiency; B) Jet velocity and jet-to-plate distance. The data represent averages from five repeats and error bars represent one standard deviation

REF: Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

7 Specific Aim VII: *Field evaluation of the portable bioaerosol samplers*

This part of the study is focused on the performance of seven portable microbial impactors when sampling culturable bacteria and fungi in indoor and outdoor environments. The performance of portable samplers in both environments was compared to those of a single stage Andersen-equivalent impactor and gelatin filter loaded into Button Aerosol Sampler.

7.1 Experimental methods

In this part of the study, we used a one-stage Andersen impactor (N-6)—equivalent microbial sampler, BioStage impactor (SKC Inc., Eighty Four, PA) as our reference sampler. BioStage impactor is the replicate of the last stage of Andersen six-stage impactor, with a cutoff size of 0.65 μm (RTI, 2004) and was used with 50mL agar. In addition, a Button Aerosol Sampler (SKC Inc., Eighty Four, PA) coupled with a gelatin filter was also included to test the performance of gelatin filter when sampling culturable microorganisms in field environments. The Button Aerosol Sampler adheres to the ACGIH (American Conference of Governmental Industrial Hygienists) inhalable particle convention (Aizenberg et al., 2000) while the gelatin filter was found to provide high collection efficiency and nutrient rich environment for the microorganisms (Clark et al., 2005).

Agar plates were prepared using trypticase soy agar (Becton, Dickson and Company, Sparks, MD) for bacteria and sabouraud dextrose agar (Becton, Dickson and Company, Sparks, MD) for fungi. Manufacturer-prepared agar strips and plates were used for the RCS High Flow and Millipore Air Tester, respectively. The agar nutrient types for these two samplers were the same as for other samplers when sampling bacteria and fungi, respectively.

Air samples were simultaneously collected by all nine samplers in an outdoor environment nearby office building and in a residential apartment. The sampling in each environment was repeated twice about 5 days apart. During each sampling day and for each environment, sampling of bacteria and fungi was repeated five times. The monitored microorganisms included all bacteria and fungi capable of growing on the respective collection media.

During the experiments, all samplers were placed in the same sampling environment, about 0.3 m away from each other, and at a height of 1.1 m above the ground or floor. The tested samplers have different sampling flow rates depending on the model, typically 100 L/min or above. BioStage impactor has a standard sampling flow rate of 28.3 L/min as recommended by the manufacturer. The recommended sampling flow rate for the Button Aerosol Sampler is 4 L/min; however, at this sampling flow rate, a sampling time of 30-60 min is needed to achieve a sample volume comparable to that of portable samplers. A long sampling time is likely to affect the culturability of the collected microorganisms because of the desiccation effect. Therefore, to decrease the needed sampling time, the sampling flow rate of the Button Aerosol Sampler with gelatin filter was increased to 10 L/min. Hauck (Hauck et al., 1997) have shown that 10 L/min sampling flow rate worked well for Button Aerosol Sampler. For seven portable microbial samplers and the BioStage impactor, the sampling time was limited to 5 min and Button Aerosol Sampler with gelatin filter was operated for 15 min. The humidity level and temperature were measured by Traceable Hydrometer (Fisher Scientific, Pittsburgh, PA). In addition, in all environments, the particle concentration levels in different size ranges were simultaneously monitored for the entire sampling periods by an Optical Particle Counter (OPC) (model

1.108, Grimm Technologies, Douglasville, GA). Five repeats were conducted for each sampling condition.

After sampling, all impactor agar plates were directly incubated at room temperature for at least 48 hours for bacteria and at least 72 hours for fungi. The gelatin filter was removed from the Button Aerosol Sampler and dissolved into 2 mL autoclaved Milli-Q water and aliquots of 200 μL suspension were plated onto the agar plates in triplicate.

The culturable bacterial and fungal concentrations both in indoor and outdoor environments were determined using the following equation:

$$C_{CFU} = \frac{N_{CFU}}{Q \times t} \tag{7.1}$$

where C_{CFU} is the culturable microorganism concentration measured by a test sampler, gelatin filter or the reference sampler BioStage impactor; N_{CFU} is the statistically corrected number of CFU (Feller, 1950) recovered by a sampler; Q is the sampling flow rate of the sampler, and t is the sampling time used for each sampler. For the gelatin filter, a factor of 10 was applied to account for the dilution of the sample during plating.

The portable samplers' ability to enumerate airborne microorganisms was compared to that of the BioStage impactor during the same repeat in the same environment. This performance comparison was quantified by the Relative Overall Efficiency, R_{EFF}, which was calculated using the following equation:

$$R_{EFF} = C_{Test} / C_{BioStage} \tag{7.2}$$

where C_{Test} is the culturable microorganism concentration measured by the test samplers including the Button Aerosol Sampler with gelatin filter, and $C_{BioStage}$ is the concentration measured by the BioStage impactor. Five R_{EFF} values were obtained for each sampler in each test environment when sampling bacteria and fungi. The results are presented as averages and standard deviations of those five repeats.

7.2 Statistical analysis

Two-way ANOVA tests were performed to analyze the relative overall efficiency, R_{EFF}, as a function of sampler model for different sampling environments, bioaerosol species (bacteria and fungi) and sampling days. The Bonferroni multiple comparison test was used to perform pairwise comparisons between the samplers. This test was chosen because it adjusts the observed significance level for multiple comparisons. In addition, we applied single and multiple linear regression to analyze the pooled R_{EFF} data as a function of two predictors (independent variables): sampler cut-off size and jet-to-plate distance. The p-values less than 0.05 indicated statistical significance in all tests.

7.3 Measured particles number distribution

The particle number distributions in the sampling environments were monitored using an Optical Particle Counter (OPC). The particles measured by the OPC include both biological and non-biological fractions. The particle concentration was normalized over the size channel width of the OPC as shown in Figure 7.1. Particles of less than 0.5 μm had the highest distribution levels, about 90% or above, both for indoor and outdoor environments, typically ranging from 10^3 to about 10^5 particles per liter of air as observed. For particles of larger than 0.5 μm, their distribution levels were relatively lower, ranging from 10^2 to 10^3 particles per liter. Higher particle concentration and variability were observed

for each specific size range in indoor environments compared to the outdoor environments. This particle concentration difference between indoor and outdoor environments could be attributed to the indoor activities which have been shown to resuspend a substantial number of particles (Thatcher and Layton, 1995). In general, both indoor and outdoor environments share similar size distribution profile after the normalization. The peaks observed around 2 μm in Figure 7.1 both for indoor and outdoor environments were caused by the narrower size channel after the normalization.

7.4 Performance when sampling bacteria

Figure 7.2 shows the Relative Overall Efficiency of the microbial samplers when sampling indoor bacteria during two separate days. The y-axis represents the R_{EFF} value for each sampler, and x-axis indicates the tested samplers and their respective cutoff sizes. Cutoff size indicates aerodynamic size of the particles at which 50% collection efficiency is achieved. These values were determined earlier research (Chapter 5). The gelatin filter does not have a cutoff size *per se*, but we observed a physical collection efficiency of about 100% when sampling *Pseudomonas fluorescens* (aerodynamic size 0.6 μm) (Chapter 6). Therefore, we indicated its cut-off size as < 0.6 μm.

Experimental data shown in Figure 7.2 indicate that for both sampling days the mean values of the Relative Overall Efficiency, R_{EFF}, for test samplers including gelatin filter were lower than unity, i.e., they recovered lower concentrations of culturable bacteria compared to the BioStage impactor. The airborne culturable microorganism concentrations measured by the BioStage impactor were 185 and 84 CFU/m^3 for Day #1 and Day #2, respectively. Among all the test samplers, RCS High Flow, MAS-100, SAS Super 180 and Millipore Air Tester had the higher mean values compared to other samplers. The mean R_{EFF} of the gelatin filter and SMA sampler were the lowest among the test samplers during both days of sampling. Since our primary goal was to analyze performances of the portable impactors and compare them against the BioStage, the results of the gelatin filter were excluded from most of the statistical analyses involving portable impactors. In addition, high variability of the gelatin filter's R_{EFF} values due to its high detection limit was observed in most of the experiments and it would have affected the results of statistical analysis. The gelatin filter's performance was separately compared against the BioStage impactor.

Two-way ANOVA analysis of the portable impactors indicated that the effects of sampler model and sampling day were statistically significant ($p<0.0001$). Multiple pair-wise comparisons using Bonferroni statistics with the BioStage as a reference showed that during Day #1, SMA, BioCulture and Microflow were statistically different (lower) compared to the BioStage. During Day #2, all samplers, except MAS-100, were statistically different (lower) compared to the BioStage. When data from both days were pooled together, only the MAS-100 was not statistically different from the BioStage, while the R_{EFF} values of other impactors were below unity. Multiple pair-wise comparisons of the pooled portable impactor data (BioStage not included) showed that the SMA sampler was different (lower) compared to the SAS Super 180, the Millipore Air Tester and the MAS-100. The rest of the pairwise comparisons were not statistically significant. The R_{EFF} value of the gelatin filter was statistically below unity for both days ($p=0.004$).

The statistically significant effect of sampling day indicates that indoor bacterial composition and/or their size distribution might have changed from Day#1 to Day#2. In general, a similar trend of the

Relative Overall Efficiencies of portable samplers relative to the BioStage impactor was observed during Day #1 and Day #2.

Figure 7.3 shows the Relative Overall Efficiencies of microbial samplers when sampling outdoor bacteria during two separate days. Among the portable impactors, the RCS High Flow and the MAS-100 had the highest mean R_{EFF} values during Day #1, while MAS-100 and BioCulture had the highest mean values during Day #2. In fact, the mean R_{EFF} values of the MAS-100 were above unity for both days, i.e., it enumerated higher average microorganism concentration compared to the BioStage. Outdoor bacteria concentrations as measured by the BioStage impactor were 155 and 114 CFU/m^3 for Day#1 and Day#2, respectively. For both days, the Millipore Air Tester and the SMA sampler had the lowest mean R_{EFF} values among the portable impactors. During Day #2, the gelatin filter also had the R_{EFF} value above unity. Visually, similar trend of the Relative Overall Efficiency of portable samplers was observed for Day #1 and Day #2.

Two-way ANOVA analysis of portable impactor data in Figure 7.3 indicated that there was a statistically significant sampler effect ($p=0.006$), but not sampling day effect ($p=0.73$). When analyzed separately for each day, there was a statistically significant difference in the R_{EFF} values among samplers for Day #2 ($p=0.033$), but not for Day #1 ($p=0.19$). Multiple pairwise comparisons using Bonferroni statistics and BioStage as a reference showed that none of the portable samplers were statistically different from the BioStage during both days. When portable sampler data from both days were pooled together and compared against the BioStage, only the Millipore Air Tester was found to be statistically different (lower). Multiple pairwise comparisons during each individual day showed that none of the samplers was statistically different from the BioStage. Multiple pairwise comparisons of the portable sampler data (no BioStage included) showed that MAS-100 was different (higher) compared to the Millipore Air Tester. The R_{EFF} value of gelatin filter was different from unity during Day#1 ($p=0.0012$), but not during Day#2 ($p=0.58$).

In general, the Relative Overall Efficiency of the Millipore Air Tester has decreased when sampling bacteria outdoors compared to indoor sampling, while the R_{EFF} values for the rest of the portable samplers have increased for outdoor sampling compared to indoor sampling.

Different from indoor sampling, when sampling outdoors, there was no statistically significant sampling day effect. This result indicates that the outdoor bacterial composition and/or size distribution remained relatively constant during two days of sampling.

BioCulture and Microflow have cutoff sizes of 7.0 and 8.8 μm, respectively, which are higher compared to other samplers (Chapter 5). These samplers were also shown to have relatively lower overall performances compared to other portable samplers when sampling laboratory-aerosolized bacteria (Chapter 6). In contrast, in outdoor environment, the R_{EFF} values of these two samplers were not different from samplers with lower cutoff sizes. It is likely that in a field environment bacterial species are attached to larger particles or are present as agglomerates, for which BioCulture and Microflow have sufficiently high physical collection efficiencies. In addition, their collection efficiencies begin to increase for particles larger than 3μm (Chapter 5). It was shown that most of the bacteria present in the natural environments are 3 μm or larger (Lighthart, 1997). This large size of bacterial species substantially increases the efficiency of their collection by most of the samplers tested.

For sampling of bacteria, the Millipore Air Tester and the SMA had lower mean R_{EFF} values than could be predicted by their cut-off sizes. This phenomenon will be discussed later.

Gelatin filter was observed to enumerate some culturable outdoor and indoor bacteria, indicating that certain hardy bacterial species resistant to desiccation stress were present in the environments. High variability of the filter efficiency was observed throughout this study, which was likely due to low concentration of desiccation-resistant microorganisms in the environments. In addition, low sampling flow rate and the need to dissolve the filter into 2mL of liquid prior to cultivation have increased the detection limit and uncertainty of the results.

The average concentration of culturable bacteria measured by the BioStage in both environments and during both dates ranged from 84 to 155 CFU/m^3. These concentrations constitute a very small fraction of all airborne particles 0.5 μm and larger as shown in Figure 7.1.

7.5 Performance when sampling fungi

Figure 7.4 shows the Relative Overall Efficiencies of the microbial samplers when sampling fungal species indoors during two independent days. During both days, all of the portable samplers had average overall efficiencies below unity, i.e., they measured lower fungal concentrations compared to the BioStage. The average fungi concentration was relatively low and ranged from 68 CFU/m^3 for Day #1 to 41 CFU/m^3 for Day#2 as measured by the BioStage. Among the portable samplers, the RCS High Flow and MAS-100 had the highest mean R_{EFF} values during Day #1, while the MAS-100 and the Microflow had the highest R_{EFF} values during Day #2. The Millipore Air Tester and the SMA had the lowest R_{EFF} values during both days. Similar to the data analysis with bacteria, we concentrated the statistical analysis on the portable samples and their performance relative to the BioStage and the performance of gelatin filter was analyzed separately.

Two-way ANOVA analysis of the portable sampler data indicated that there was a statistically significant sampler effect ($p=0.006$), but not sampling day effect ($p=0.65$). Multiple pairwise portable sampler comparisons with Bonferroni statistics using the BioStage as a control showed that only the Millipore Air Tester and the SMA were statistically different (lower) compared to the BioStage during Day#1 and Day#2. When the R_{EFF} data from both days were pooled together, only the MAS-100 and the Microflow were not statistically different from the BioStage, while performance of other portable samplers was lower compared to the BioStage. Multiple pairwise comparisons of the pooled portable sampler data (BioStage not included) indicated that SMA was statistically different (lower) compared to the MAS-100, and Microflow was statistically different (higher) compared to the Millipore Air Tester and the SMA. All other pairwise comparisons were not statistically different. The R_{EFF} of gelatin filter was below unity for Day #2 ($p = 0.0006$), but not for Day #1 ($p=0.37$). However, due to variability of the data, during Day#1 it had coefficient of variation > 100%.

As indicated by the statistical analysis, the effect of the sampling day was not statistically significant on the portable sampler performance. This suggests that indoor fungi composition and/or size distribution remained relatively constant in contrast to indoor bacteria.

Figure 7.5 shows the Relative Overall Efficiencies of the microbial samplers when sampling outdoor fungi species during two separate days. During both days, the RCS High Flow and the MAS-100 had

the highest mean R_{EFF} values. In fact, the values were close to or above unity, i.e., the average fungi concentrations measured by these samplers were similar or higher than those measured by the BioStage impactor. Average airborne fungi concentrations measured by the BioStage were slightly higher compared to indoor environment: 85 and 94 CFU/m^3 for Day # and Day #2, respectively.

Two-way ANOVA analysis of the portable impactors indicated that there was a statistically significant effect by both sampling days (p=0.024) and sampler model (p=0.0001) on the relative performance of the portable samplers. Multiple pairwise comparisons with Bonferroni statistics against BioStage as control showed that none of the samplers were different from the BioStage during Day #1 (p=0.097), while during Day#2 Millipore Air Tester, SMA, and Microflow were different (lower) compared to BioStage. When data from both days were pooled together, only Millipore and SMA were statistically different (lower) compared to the BioStage.

Multiple pairwise comparisons of the pooled portable sampler data (BioStage not included) indicated that the RCS High Flow was statistically different (higher) compared to the Millipore Air tester and the SMA, while MAS-100 was different (higher) compared to the SMA. All other pairwise comparisons were not statistically different. The R_{EFF} value of the gelatin filter was below unity for both days (p = 0.003).

Statistically significant sampling day effect suggests that the outdoor fungi composition and/or size distribution was different between the two sampling days. The maximum concentration of culturable fungi in both indoor and outdoor environments as measured by the BioStage was close 100 CFU/m^3. This concentration is much lower than the concentration of airborne particles larger than 2 μm in size (Figure 7.1). Data from both bacterial and fungal sampling indicated that culturable particles constituted only a small fraction of all airborne particles during our measurements.

7.6 General Description of Portable Impactor Performance

Since one of the main goals of this study was to compare the portable impactor performance in different environments when sampling different species, for the final analysis, we analyzed all the R_{EFF} data as a function of sampler model and compared their performance against the BioStage. In this case, each sampler had a data set of 40 measurements. The mean R_{EFF} values and standard deviations for each sampler are presented in Figure 7.6. It could be seen that the MAS-100 had the mean R_{EFF} value equal to a unity – highest among the portable impactors. The mean R_{EFF} value of the RCS High Flow was approximately 0.9, while the SAS Super 180, the BioCulture and the Microflow had the mean R_{EFF} values of approximately 0.7. The Millipore Air Tester and the SMA samplers had the lowest R_{EFF} values among the portable impactors - approximately 0.4. For gelatin filter the R_{EFF} value was slightly higher: 0.46. Multiple pairwise comparisons with Bonferroni statistics against BioStage as a reference showed that among the portable impactors only the RCS High Flow and the MAS-100 were not statistically different from the BioStage, while the R_{EFF} values of all other samplers were below unity. Gelatin filter was also statistically different (lower) compared to the BioStage (p<0.0001). Multiple pairwise comparisons of the pooled portable sampler data (BioStage not included) indicated that MAS-100 was statistically different (higher) compared to all portable samplers, except RCS High Flow. The two samplers showing the lowest R_{EFF} values, SMA and Millipore Air Tester, were found to be statistically different compared to all other portable samplers. The rest of the pairwise comparisons were not statistically different. When gelatin filter was compared against the portable samplers, it was

found to be statistically different (lower) compared to the RCS High Flow, SAS Super 180, and MAS-100.

According to the Figure 7.6, the concentration of airborne microorganisms determined by the gelatin filter was approximately 46% of the concentration determined by the BioStage. Although the gelatin filter provides a more favorable environment for the microorganisms compared to the regular filters, the desiccation effect is still substantial and only hardier species are enumerated.

The data in Figure 7.6 also indicate a substantial variability of the R_{EFF} for each test sampler (coefficients of variation > 50%). This variability could be reflective of inhomogeneous microorganism distribution in the air. In addition, the measurements were performed during early Spring and the relatively low culturable microorganism concentrations as indicated by the BioStage (100 – 200 CFU/m^3) are likely to have contributed to the variability of the R_{EFF} values. On the other hand, the statistical differences among the samplers elucidated even from relatively low airborne microorganism concentrations are likely to be valid when microorganisms are present in higher concentrations.

Among the portable samplers, the RCS High Flow and the MAS-100 had the lowest cut-off sizes among the portable impactors, yielding best performances. Thus, their high relative performance could be attributed to efficient particle collection. The high relative performance of the BioStage could also be attributed to its low cutoff size, i.e., ability to collect even single bacteria. The BioCulture and the Microflow had the largest cutoff sizes (7.0 and 8.8μm), but their R_{EFF} values were close to those of the SAS Super 180, which had a cut-off size of 2.1μm. The BioCulture and the Microflow have rather low jet velocities and their collection efficiency curves begin to incline at 3μm (Chapter 5). It has been suggested that high impaction velocity might negatively affect the microorganism survival during sampling (Willeke et al., 1995). Thus, these two samplers were able to effectively collect biological particles attached to larger particles, while low jet velocity provided minimal mechanical damage to the particles. The two samplers with the lowest R_{EFF} – the Millipore Air Tester and the SMA – do not have the largest cutoff sizes, but feature the largest jet-to-plate distances among the tested impactors (Chapter 5). We speculate that impaction jets dissipate due to large jet-to-plate distances and desiccate a greater fraction of the agar surface thus providing an additional stress to the collected microorganisms. The role of the jet-to-plate distance on the performance of the portable impactors when collecting fungi was demonstrated in Chapter 6.

In an attempt to generalize the bioaerosol impactor performance (including the BioStage) we performed multiple linear regression analysis of the average R_{EFF} values using cutoff size and jet-to-plate distance as predictor variables. The analysis yielded the following model with correlation coefficient R^2= 0.83 (p= 0.012):

$$R_{EFF} = 1.24 - 0.12S - 0.042d_{50}, \qquad (7.3)$$

where the S (in mm) is the jet-to-plate distance and the d_{50} (in μm) is the cutoff size. The p-value for each variable indicated statistical significance: 0.007 and 0.03 for S and d_{50}, respectively. The single linear regression with predictor variables S and d_{50} separately yielded R^2 values of 0.53 (p=0.04) and 0.16 (p=0.32), respectively. Thus, it is evident that the inclusion of both predictor variables yielded the best linear regression result. Inclusion of other design parameters, such as jet velocity or dimensionless S/W (jet diameter over jet-to-plate distance), into the regression model was not statistically significant. In addition, humidity (40-50% for outdoor, and 70% for indoor) and temperature might affect the

ability of the sampler to maintain the culturability of the collected microorganisms, but they remained the same for all test samplers during the experiments, thus excluded from the regression as well. The Eq. 7.3 implies that the highest achievable R_{EFF} value would be 1.24. This limiting value is produced by the limited number of samplers included in the model. Data from additional bioaerosol impactors, including those that have better performance than the BioStage or its equivalents, would provide a refinement to this empirical formula. However, we believe that Eq. 7.3 could be used as an approximation in predicting overall performance of the portable microbial impactors when sampling in indoor and outdoor environments.

7.7 Discussion

This part of the study has shown that different portable microbial samplers yielded different airborne microorganism concentrations in indoor and outdoor environments. It was also found that the impactor performance in a particular environment could vary from day to day, likely due to the different airborne microorganism composition and/or size distribution. It is hoped that the data presented in this study will help field professionals and bioaerosol researchers in selecting bioaerosol samplers for their needs. It is hoped that the developed empirical formula to predict overall sampler performance will aid in designing more efficient bioaerosol samplers.

7.8 Tables and figures for Chapter 7

Table 7.1. Statistical analysis of biological performance of portable microbial impactors when sampling bacteria and fungi in indoor and outdoor environments. Only statistically significant relationships are presented in the table ($p < 0.05$ indicated statistical significance).

			Two-Way ANOVA for Sampling Day and Sampler	Statistical significance of multiple pairwise Bonferroni comparisons relative to the BioStage impactor (reference) for Day#1 and Day #2 separately		Statistical significance of multiple pairwise Bonferroni comparisons relative to the BioStage impactor (reference) when data from Day#1 and Day #2 are pooled	Statistical significance of multiple pairwise Bonferroni comparisons of the pooled portable impactor data (BioStage not included)
Bacteria	**Indoors**	Sampling day	p<0.0001	Day 1	SMA, BioCulture and Microflow different (lower)	All samplers different (lower), except MAS-100	SMA different (lower) compared to the SAS Super 180, the Millipore Air Tester and the MAS-100
		Sampler	p<0.0001	Day 2	All samplers different (lower), except MAS-100		
	Outdoors	Sampling day	p=0.73	Day 1	None different	Millipore Air Tester (lower)	MAS-100 different (higher) compared to the Millipore Air Tester
		Sampler	p=0.006	Day 2	None different		
Fungi	**Indoors**	Sampling day	p=0.65	Day 1	Millipore Air Tester and SMA different (lower)	All samplers different (lower), except MAS-100 and Microflow	The SMA different (lower) compared to the MAS-100; Microflow different (higher) compared to the Millipore Air Tester and the SMA
		Sampler	p=0.006	Day 2	Millipore Air Tester and the SMA different (lower)		
	Outdoors	Sampling day	p=0.024	Day 1	None different	Millipore and SMA (lower)	RCS High Flow different (higher) compared to the Millipore Air tester and the SMA; MAS-100 different (higher) compared to the SMA
		Sampler	p=0.0001	Day 2	Millipore Air Tester, SMA, and Microflow different (lower)		

REF: Yao M and Mainelis G: [2007] Journal of Occupational and Environmental Hygiene 4: 514-524.

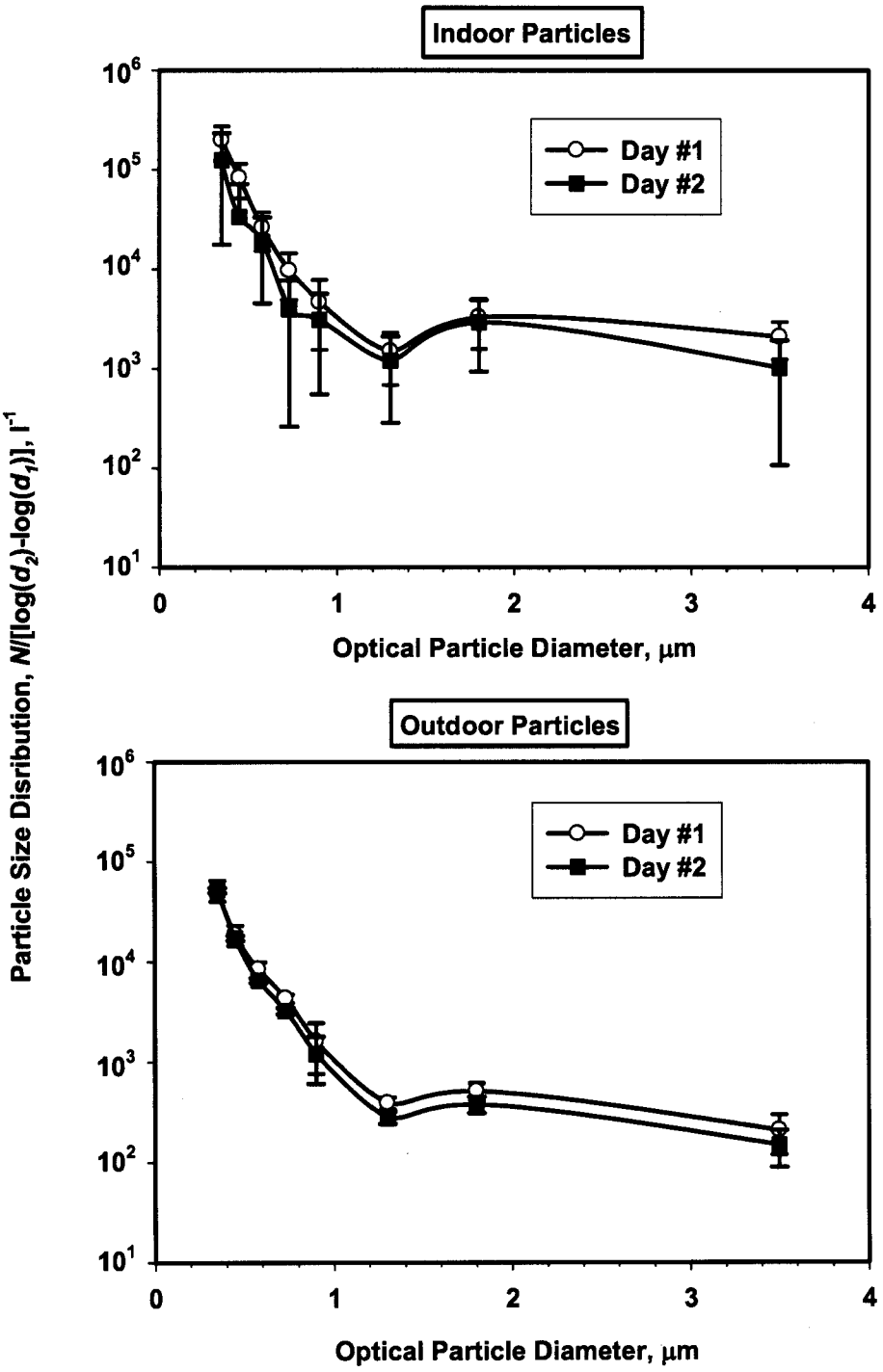

Figure 7.1. Normalized particle distributions in indoor and outdoor environments during the entire sampling periods.

REF: Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

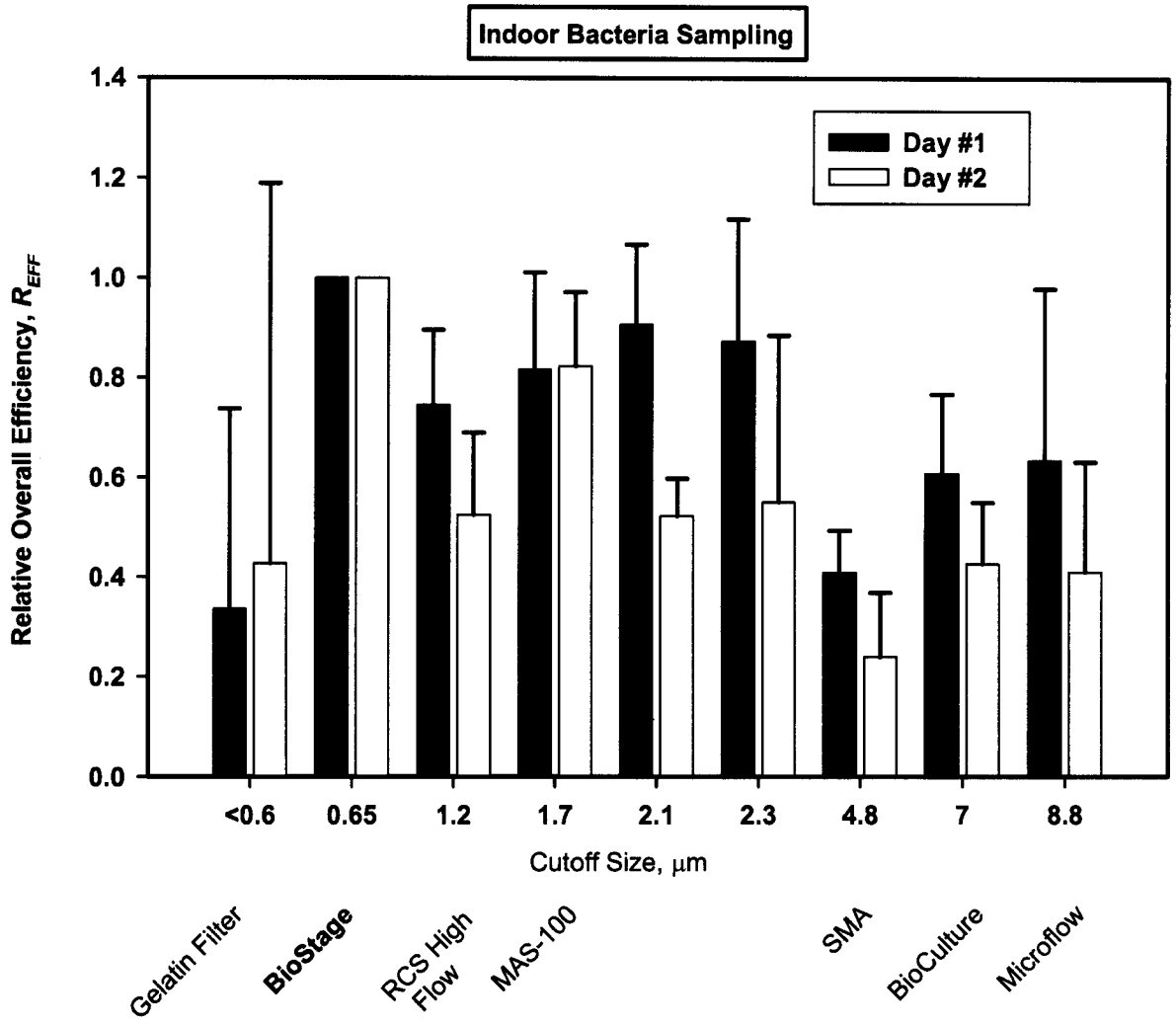

Figure 7.2. Overall performance of microbial samplers relative to BioStage impactor when sampling indoor bacteria; data represent averages of five repeats and error bars stand for one standard deviation.

REF: Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

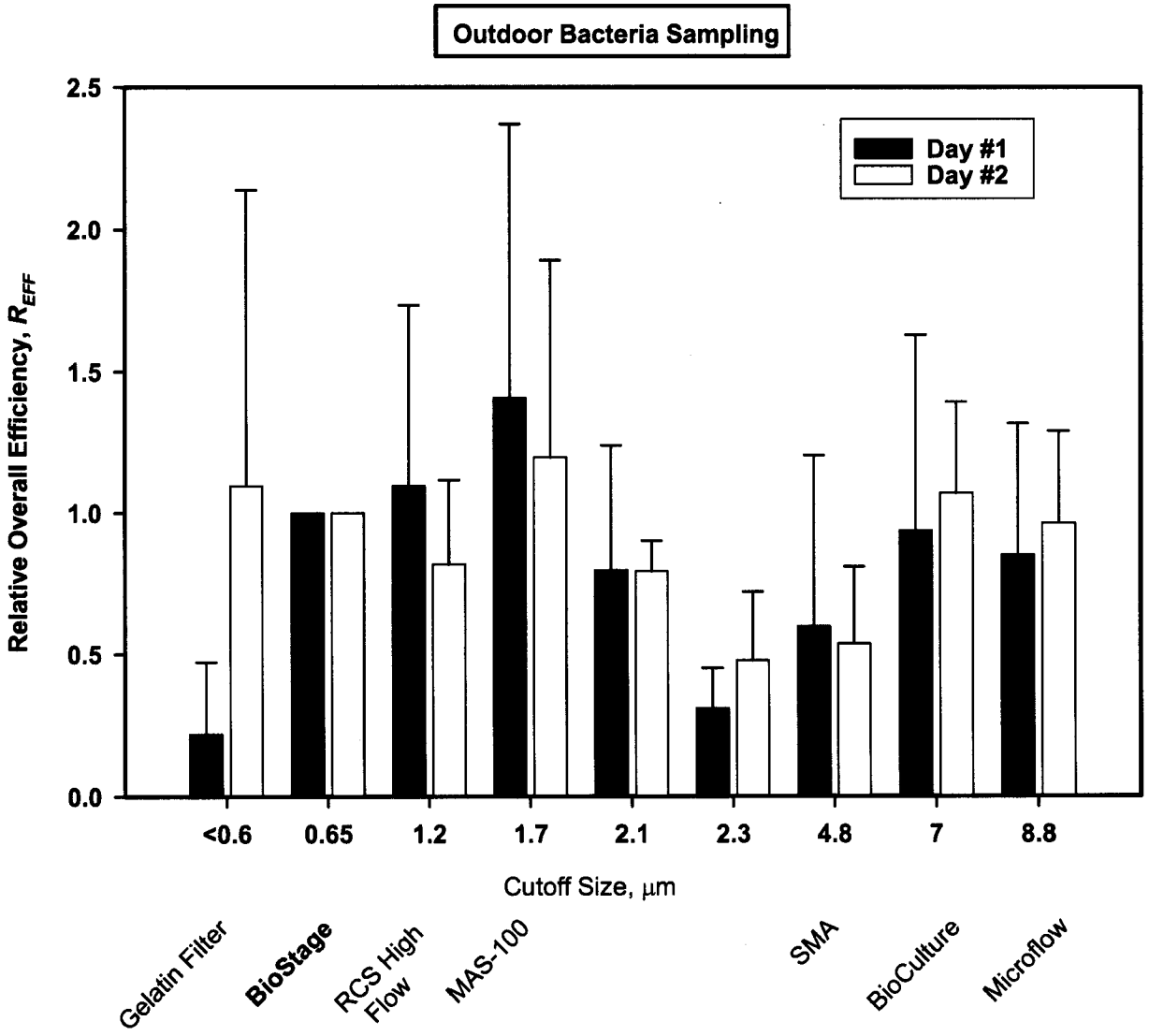

Figure 7.3. Overall performance of microbial samplers relative to BioStage impactor when sampling outdoor bacteria; data represent averages of five repeats and error bars stand for one standard deviation.

REF: Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

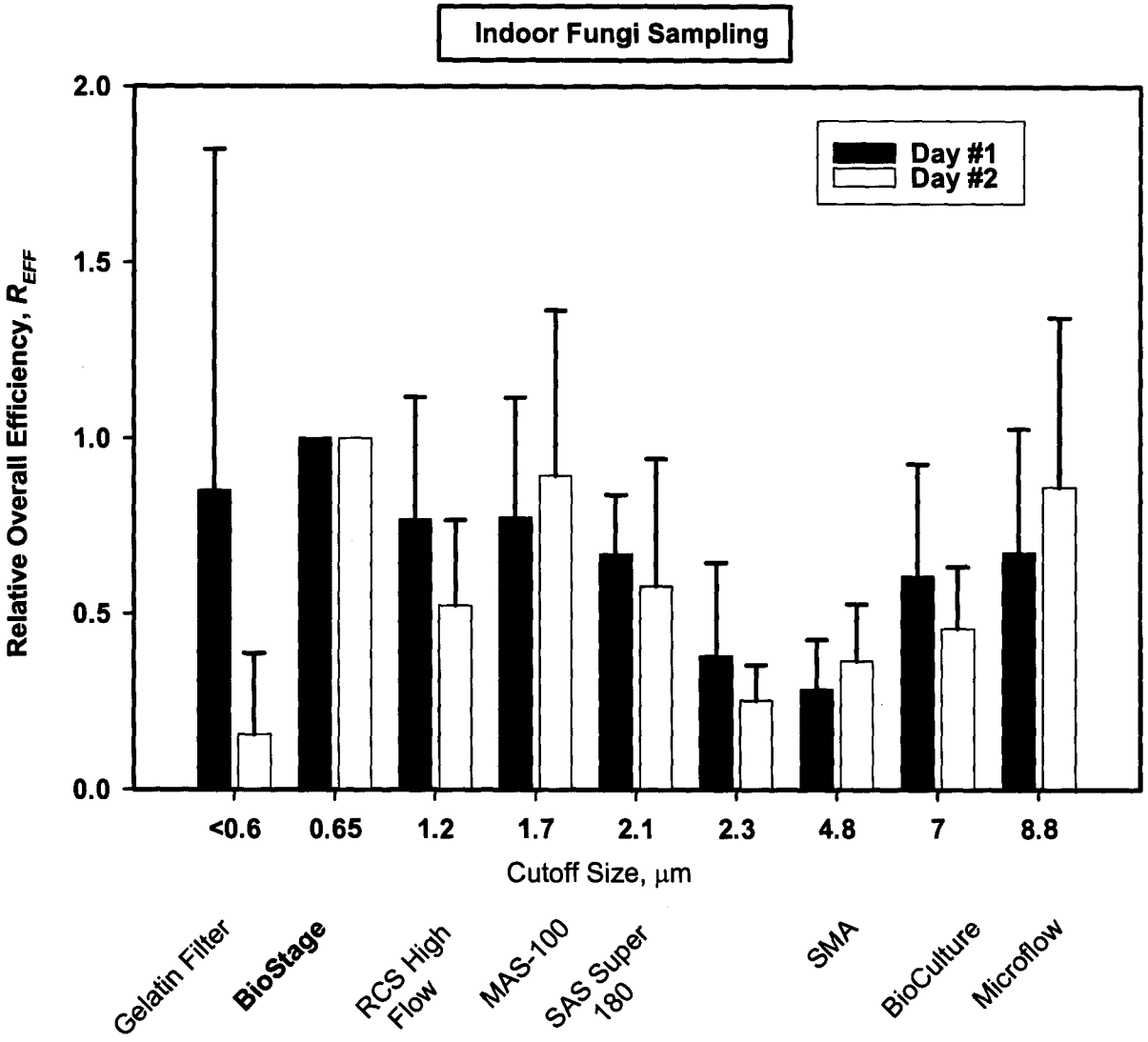

Figure 7.4. Overall performance of microbial samplers relative to BioStage impactor when sampling indoor fungi; data represent averages of five repeats and error bars stand for one standard deviation.

REF: Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

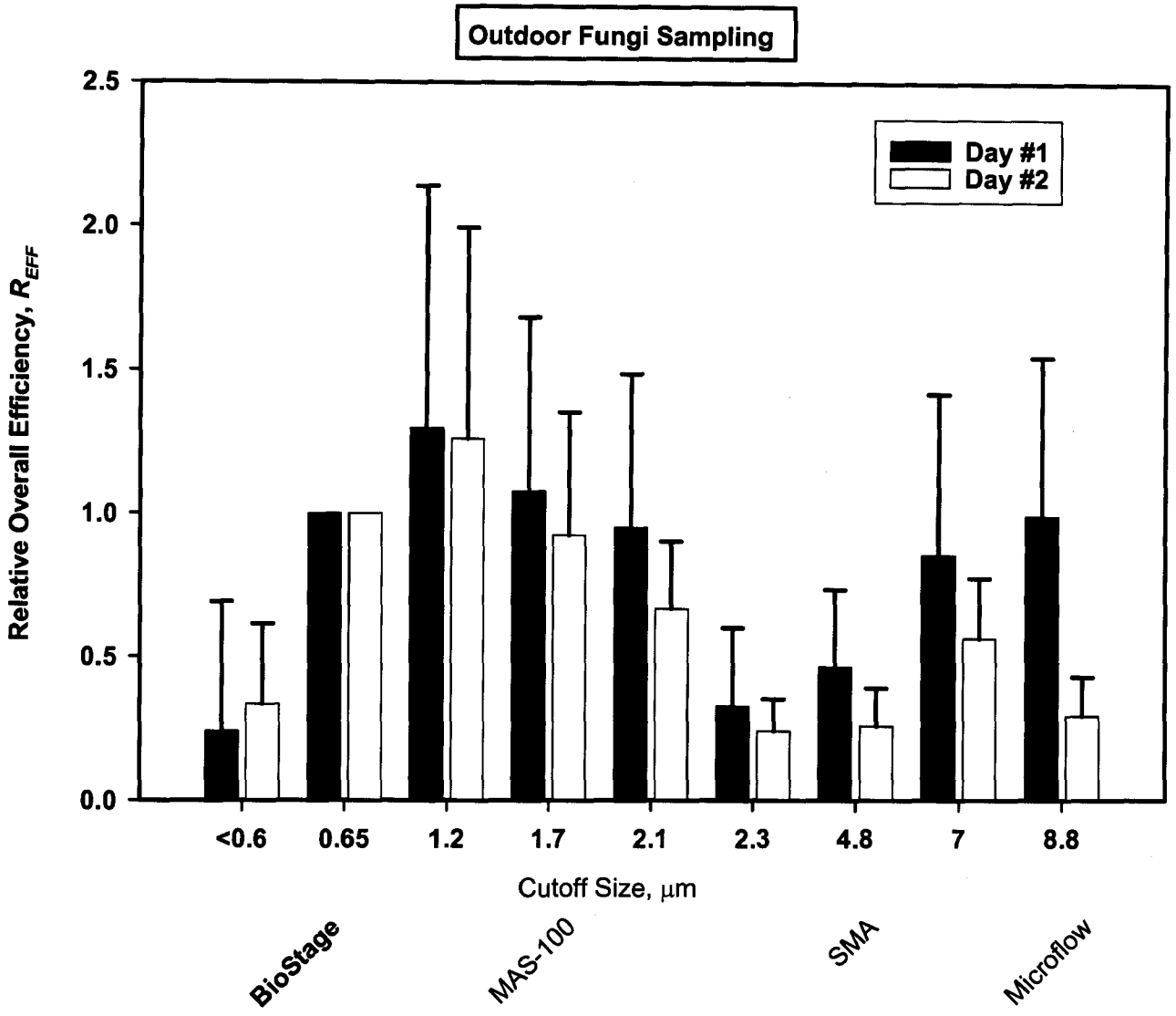

Figure 7.5. Overall performance of microbial samplers relative to BioStage impactor when sampling outdoor fungi; data represent averages of five repeats and error bars stand for one standard deviation.

REF: Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

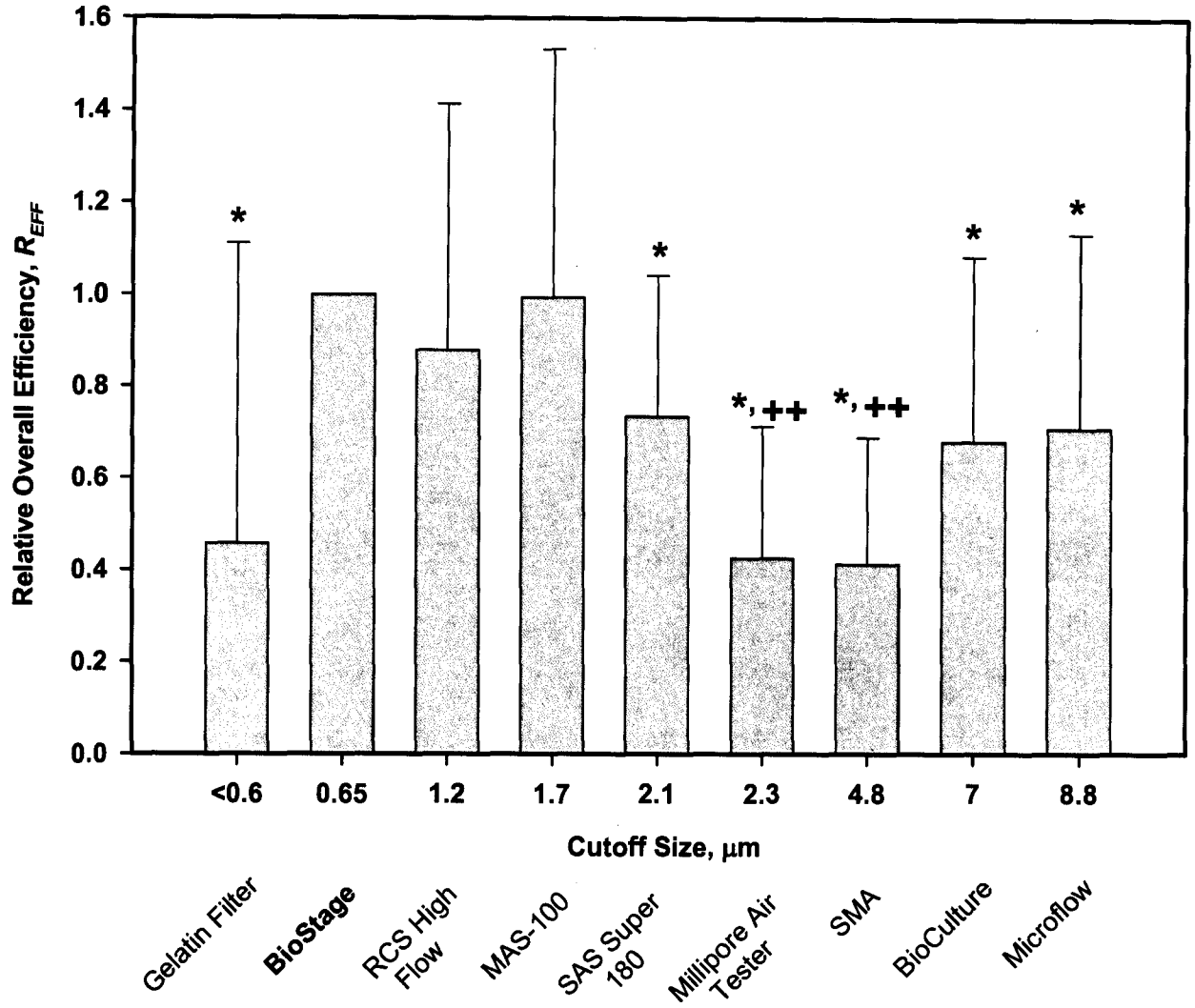

Figure 7.6. Relative overall efficiency of the bioaerosol samplers when collecting bacteria and fungi in indoor and outdoor environments.
* indicates samplers that were different statistically different from the BioStage.
++ indicates samplers that were statistically different from other portable samplers.

REF: Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

8 Specific Aim VI: *Investigation of the sampling time effect on the performance of the samplers*

In this part of the study we analyzed whether the sampling time affects the overall performance of seven portable impactors mentioned above when collecting airborne bacteria and fungi from 2 to 30 min indoors and outdoors. In addition, to better ascertain the factors responsible to likely decrease in samplers' performance with prolonged sampling time, we separately investigated the effects of desiccation damage to the already collected microorganisms as well as the effect of agar desiccation prior to collecting the microbial particles.
The amount of agar used to operate each impactor was the same as used in earlier part of this research and reflects either a manufacturer's recommendations or was determined to yield lower cutoff sizes (Chapter 5). In addition to portable impactors, we used two one-stage Andersen impactor (N-6)—equivalent microbial samplers, BioStage impactor (SKC Inc., Eighty Four, PA). One of them served as a reference sampler, while the other was operated in parallel to the portable impactors. The BioStage impactor is the replicate of the last stage of Andersen six-stage impactor, which has a cutoff size of 0.65 μm (RTI, 2004) and was used with 50 mL agar. The Laboratory and field research presented in Chapters 6 and 7 clearly indicated that the biological collection efficiency of an inhalation-based reference sampler (Button Aerosol Sampler) equipped with gelatin filter is lower than that of a traditional reference sampler (Andersen-equivalent impactor). In addition, sensitive bacterial species (*P. fluorescens* and *E. coli*) were not detected when sampling in a laboratory for a short period of 5 minutes. Therefore, the inhalation-based Button Aerosol Sampler was not included in this part of the study and we focused on the sampling time effect on the performance of portable impactors.

8.1 Sampling protocol and determination of samplers' performance

Since it is not completely clear whether the desiccation of already collected microorganisms or desiccation of agar prior to collecting the microorganisms is a more substantial causative factor for a decrease in sample CFUs, the sampling procedures were divided into three separate protocols.

Protocol A. The bacterial and fungal samples were collected indoors with all test impactors operating simultaneously and collecting samples for different sampling periods: t_S = 2, 5, 10, and 30 minutes. During all four different sampling periods, the reference BioStage impactor collected samples for t_R = 2 minutes only. Overall, nine replicates over several days for both bacteria and fungi were collected for each sampling time and environment. The collected samples were incubated at room temperature and the formed Colony Forming Units (CFUs) were counted after 24, 48 and 72 hours. The final number of CFUs was adjusted for positive-hole correction and fungal and bacterial concentrations were calculated as CFUs/m^3. Tryptic soy agar (TSA) (Becton Dickinson Microbiology Systems, Cockeysville, MD) was used for collection of bacteria, while malt extract agar (MEA) (Becton Dickinson Microbiology Systems) was used to collect fungi. To determine the effect of sampling time on the performance of each sampler, the *Concentration Ratio A*, $CR\_A(t_S, i)$, was determined as

$$CR\_A(t_S, i) = \frac{C(t_S, i)}{C_R(t_S = 2\,\text{min})}, \qquad (8.1)$$

where $C(t_S, i)$ is a concentration determined by a test sampler i (seven portable samplers and BioStage impactor) during sampling time t_S, and the C_R is a concentration measured by a reference sampler. This procedure was applied for both bacteria and fungi and for each replicate separately. The preliminary

outdoor sampling experiments indicated that for most of the samplers, sampling times above 5 min caused CFU overload, therefore this procedure was not performed outdoors.

Protocol B was designed to investigate the effect of desiccation on already collected microorganisms. Here, the seven portable samplers and two BioStage impactors were operated in parallel outdoors. Each test sampler i and the reference sampler collected bacteria or fungi simultaneously for $t_{COLL} = 2$ min, then were transferred indoors and operated in a particle-free environment for t_S–t_{COLL}, where t_S = 2, 5, 10, and 30 min. Thus, the minimum exposure to particle-free air was 0 min and maximum 28 min. The reference sampler operated for $t_{COLL} = 2$ min outdoors only. The particle free environment was ensured by operating the impactors inside a Class II biosafety cabinet (Nuaire Inc., Plymouth, MN), while the temperature and relative humidity corresponded to comfortable room conditions. The agar types, incubation conditions and counting procedures were the same as in the protocol A. The effect of microorganism desiccation was determined as the *Concentration Ratio B*, *CR_B*:

$$CR\_B(t_{COLL}+[t_S-t_{COLL}],i)=\frac{C(t_{COLL}+[t_S-t_{COLL}],i)}{C_R(t_{COLL}=2\,\text{min})}, \qquad (8.2)$$

Where $C(t_{COLL}+[t_S-t_{COLL}],i)$ is a concentration of bacteria or fungi determined by a test sampler after sampling the biological particles for $t_{COLL} = 2$ min and then sampling particle-free air for t_S–t_{COLL} time period and the C_R is a concentration measured by a reference sampler. The calculations were performed for bacteria and fungi and for each of the nine repeats separately. The repeats were spread over several days. Due to low microorganism concentrations indoors, the sampling of organisms was performed outdoors only.

Protocol C was designed to investigate the effect of desiccating agar prior to collecting the microorganisms. Here, the seven portable samplers and a test BioStage impactor were operated simultaneously in a particle-free environment for t_S–t_{COLL}, where t_S = 2, 5, 10, and 30 min and $t_{COLL} = 2$ min. The particle-free environment was the same as in the protocol B. After sampling particle-free air, the samplers were transferred outdoors and simultaneously with the reference sampler collected samples for $t_{COLL} = 2$ min. Thus, before collecting actual samples, the minimum exposure to particle-free air was 0 min and maximum 28 min. The reference sampler has not been exposed to a particle-free air. The agar types, incubation conditions and counting procedures were the same as in the protocol A. The effect of agar microorganism desiccation was determined as the *Concentration Ratio C*, *CR_C*:

$$CR\_C([t_S-t_{COLL}]+t_{COLL},i)=\frac{C([t_S-t_{COLL}]+t_{COLL},i)}{C_R(t_{COLL}=2\,\text{min})}, \qquad (8.3)$$

Where $C([t_S-t_{COLL}]+t_{COLL},i)$ is a concentration of bacteria or fungi determined by a test sampler after sampling particle-free air for t_S–t_{COLL} time period and then sampling the biological particles for t_{COLL} = 2 min and the C_R is a concentration measured by a reference sampler (not exposed to particle-free air). The calculations were performed for bacteria and fungi and for each of the nine repeats separately. The repeats were spread over several days. Due to low microorganism concentrations indoors, the sampling of organisms was performed outdoors only.

8.2 Statistical analysis

Two-way ANOVA tests were performed to analyze the *Concentration Ratios* (*CR_A*, *CR_B*, and *CR_C*) as a function of sampler model and sampling time. The p-values less than 0.05 indicated statistical significance in all tests.

8.3 The effect of sampling time on the performance of the portable impactors

The indoor concentration ratios for bacteria and fungi are presented in Figures 8.1 and 8.2. Here the test samplers were operated for different sampling times and the obtained microorganism concentrations were compared to those measured by the reference BioStage sampler operated for t_s=2 min (Protocol A). The indoor bacterial and fungal concentrations measured by the reference BioStage impactor was generally below 200 CFU/m^3.

For the sampling of bacteria and sampling time t_s=2 min, the BioStage impactor had the highest average *Concentration Ratio* of 0.9, while the SAS-180 had an average ratio of 0.7. The *Concentration Ratios* of all test samplers gradually decreased as the sampling time increased. In fact, the average *CR_A* of all samplers sampling bacteria for t_s=2 min was 0.45 and gradually decreased to 0.2 as sampling time increased to 30 min. When sampling fungi for t_s = 2min, the test BioStage impactor had the highest *Concentration Ratio* of 1.25 with MAS-100 being second with the Concentration Ratio of 1.02. The same ratios for the SAS-180 and RCS High Flow were approximately 0.8, and for the Millipore Air Tester 0.4. These ratios gradually declined as the sampling time increased. Somewhat surprising was very low concentration ratios exhibited by the SMA Micro Portable, the Microflow and the BioCulture samplers. However, it is consistent with their underperformance compared to other samplers (Chapters 6 and 7). The average *CR_A* of all samplers sampling fungi for t_s=2 min was 0.45 and gradually decreased to 0.29 as sampling time increased to 30 min. For both the sampling of bacteria and fungi, the effect of sampling time t_s and the impactor model were statistically significant ($p<0.0001$). This result does indicate that the recovery of airborne bacteria and fungi by microbial impactors decreases as sampling time increases.

In the next set of experiments (Protocol B) we examined the possible effects of desiccation on the already collected microorganisms as the sampling continues. The results for outdoor sampling of bacteria and fungi are presented in Figures 8.3 and 8.4, respectively. Here the microorganisms were collected outdoors by each sampler for t_{COLL}=2 min and then exposed to particle-free air for, resulting in exposures of 0, 3, 8, and 28 min. According to the reference sampler, the outdoor bacterial concentrations ranged from 200 to 2,000 CFU/m^3, while fungal concentrations ranged from 600 to 8,000 CFU/m^3. As could be clearly seen from Figure 8.3 and 8.4, *Concentration Ratios* steadily and consistently declined for each sampler and for each microorganism type as we went from no desiccation (t_s = 2 min, t_s-t_{COLL}=0 min) to 28 min of exposure to particle-clean air (t_s = 30 min, t_s-t_{COLL}=28 min). In fact, the *CR_B* for bacteria averaged for all samplers decreased from 0.57 at t_s=2 min to 0.03 at t_s=30 min. For fungi, the situation was similar and the *CR_B* averaged for all samplers decreased from 0.3 to 0.03 as t_s was increased from 2 min to 30min. Two-way ANOVA analysis for bacteria and fungi indicated that that both the effect of sampling time t_s and the impactor model were statistically significant ($p<0.0005$). In fact, the p-values for time effect were extremely small: $<10^{-39}$. The results obtained here clearly indicate that once microbial particles are collected, they are prone to damage as the sampling continues and they are exposed to air flow. The damage and inactivation are likely due to dehydration (Hensel and Petzoldt, 1995). If the sampling continues for a long time, their recovery can be reduced by a factor of 10. Even short exposure of 3 min can reduce their recovery by a factor of 2.

In the third set of experiments (Protocol C), we investigated the recovery of microorganisms when collection media is subjected to desiccation prior to sampling the organisms. The results for outdoor sampling of bacteria and fungi are presented in Figures 8.5 and 8.6, respectively. Here, each of the test

samplers collected particle-free air for t_s-t_{COLL} (t_{COLL}=2 min) resulting in particle-free air sampling of 0, 3, 8, and 28 min before collecting bacterial of fungal samples outdoors for t_{COLL}=2 min. The reference sampler collected microorganisms only for t_{COLL}=2 min and was not exposed to particle-free air. According to the reference sampler outdoor bacterial concentrations ranged from 200 to 1,000 CFU/m^3, while fungal concentrations were approximately 3,000 CFU/m^3. As could be clearly seen from Figures 8.5 and 8.6, the *Concentration Ratios* steadily and consistently declined for each sampler and for each microorganism type as collection media exposure to particle-free air was increased from 0 min to 28 min prior to sampling the organisms. The *CR_C* for bacteria averaged for all samplers decreased from 0.77 at t_s=2 min to 0.04 at t_s=30 min. For fungi, the situation was similar and the *CR_C* averaged for all samplers decreased from 0.4 to 0.02 as t_s was increased from 2 min to 30 min. Two-way ANOVA analysis for bacteria and fungi indicated that that both the effect of sampling time t_s and the impactor model were statistically significant ($p<0.05$). The p-values for time effect were also very small: $<10^{-17}$. The results obtained here clearly indicate that if collection media (area under the impaction nozzles) is exposed to air prior to collecting the microorganisms, the microorganism recovery could be greatly reduced. Sampling of particle-free air for 3 min can reduce the recovery of bacteria and fungi by a factor of 2-3. If the exposure to particle-free air is increased to 28 min, the recovery could be reduced by a factor of 20 compared to no exposure condition. The likely reason for such a dramatic decrease in microorganism recovery in the desiccation and hardening of agar under the impaction jet which results in particle bounce and reduced collection efficiency as suggested by earlier studies (Juozaitis et al., 1994). Even if the microorganisms do not bounce off and are deposited on agar they would have reduced access to nutrients due to hardening of agar surface.

8.4 Discussion

The data presented here clearly indicate that recovery of airborne microorganisms collected using portable impactors as well as traditional stationary impactors decreases as the sampling time increases. Once the organisms are deposited onto the collection media they are being exposed to air flow over them as the sampling continues causing their dehydration and inactivation. If an area under the impaction nozzle is exposed to air prior to depositing the organism it becomes desiccated and hard resulting in a microorganism bounce or reduced access to nutrients. In fact, visual observation of the collection media after prolonged sampling time revealed clear indentations under the sample nozzles. These indentations result in an increased in jet-to-plate distance which results in decreased collection efficiency (Chapter 6). The extent of damage to the organisms would also depend on the relative humidity of the air sampled (Thompson et al., 1994). Therefore, the data obtained here for different sampling times should not be used for their absolute value as sampling circumstances and conditions may differ from investigation to investigation, but as an indication that prolonged sampling times would decrease the culturability of the collected microorganisms which would negatively affect the accuracy of a microbial investigation.

Overall, the results of this study are consistent with those of earlier studies indicating the longer sampling times would result in decreased microorganism recovery as indicated by Juozaitis et al. (1994) and Hensel and Petzoldt (1995). The data obtained here indicate that the sampling time should be as short as possible to avoid the reduced microorganism recovery. Our observation is different from the laboratory study by Li and Lin (1999) who indicated that sampling times up to 40 min did not have a significant effect of microorganism recoveries. The difference in outcomes could be explained by the differences in experimental protocols and microorganisms used. Li and Lin (1999) investigated *B.*

subtilis and *E. coli* bacteria in a laboratory study. *B. subtilis* is a hardy organism (Sneath, 1986) resistant to many adverse conditions and probably was not affected by desiccation due to long sampling times. *E. coli* on the other hand is a sensitive organism (An et al., 2006) and was most likely affected by desiccation over prolonged sampling periods. However, the reference in the Li and Lin (1999) study was the concentration of colonies in the Collison nebulizer suspension. When sensitive microorganisms are being recirculated in a Collison nebulizer and subjected to shear stress as their aerosolization continues, their viability decreases and amount of injury increases. This phenomenon was demonstrated with sensitive bacteria *P. fluorescens* in other studies (Reponen et al., 1997; Mainelis et al., 2005). Since the culturability of both the reference bacteria and the airborne bacteria decrease, the effect of prolonged sampling time becomes obscured.

The data obtained here show that for all testing protocols, the sampler model and sampling time were statistically significant. It has also been shown that that both the desiccation of the collection media and the desiccation of the already collected organisms play a role in a decreased recovery with increased sampling time. The results of our investigation suggest that when impactors are used for the collection of airborne bacteria and fungi, sampling times should be reasonably short to minimize under-representation of airborne microorganism concentration.

8.5 Figures for Chapter 8

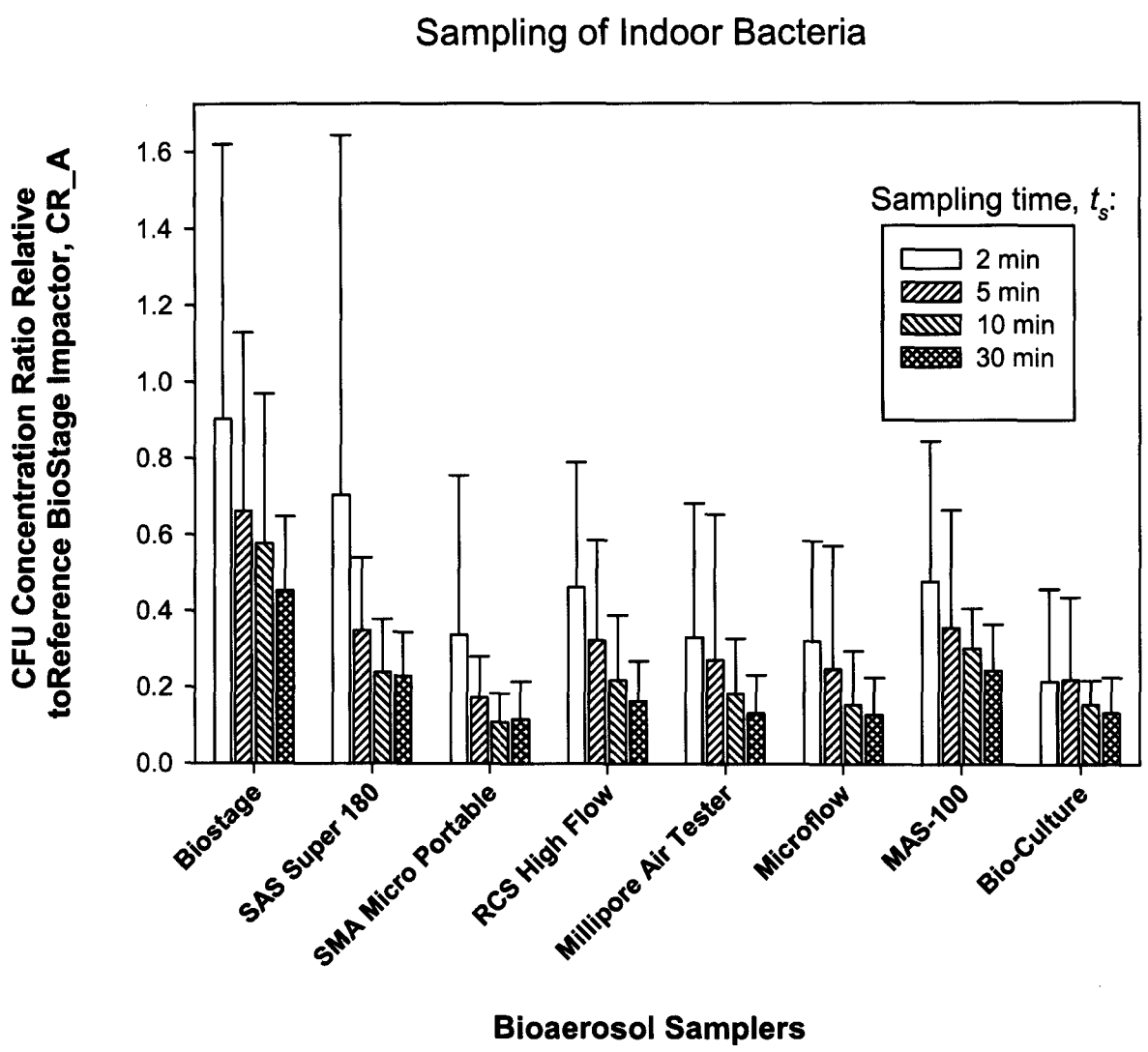

Figure 8.1. Relative performance of portable impactors as a function of sampling time, t_S, when sampling bacteria indoors.

REF: Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology *to be submitted*.

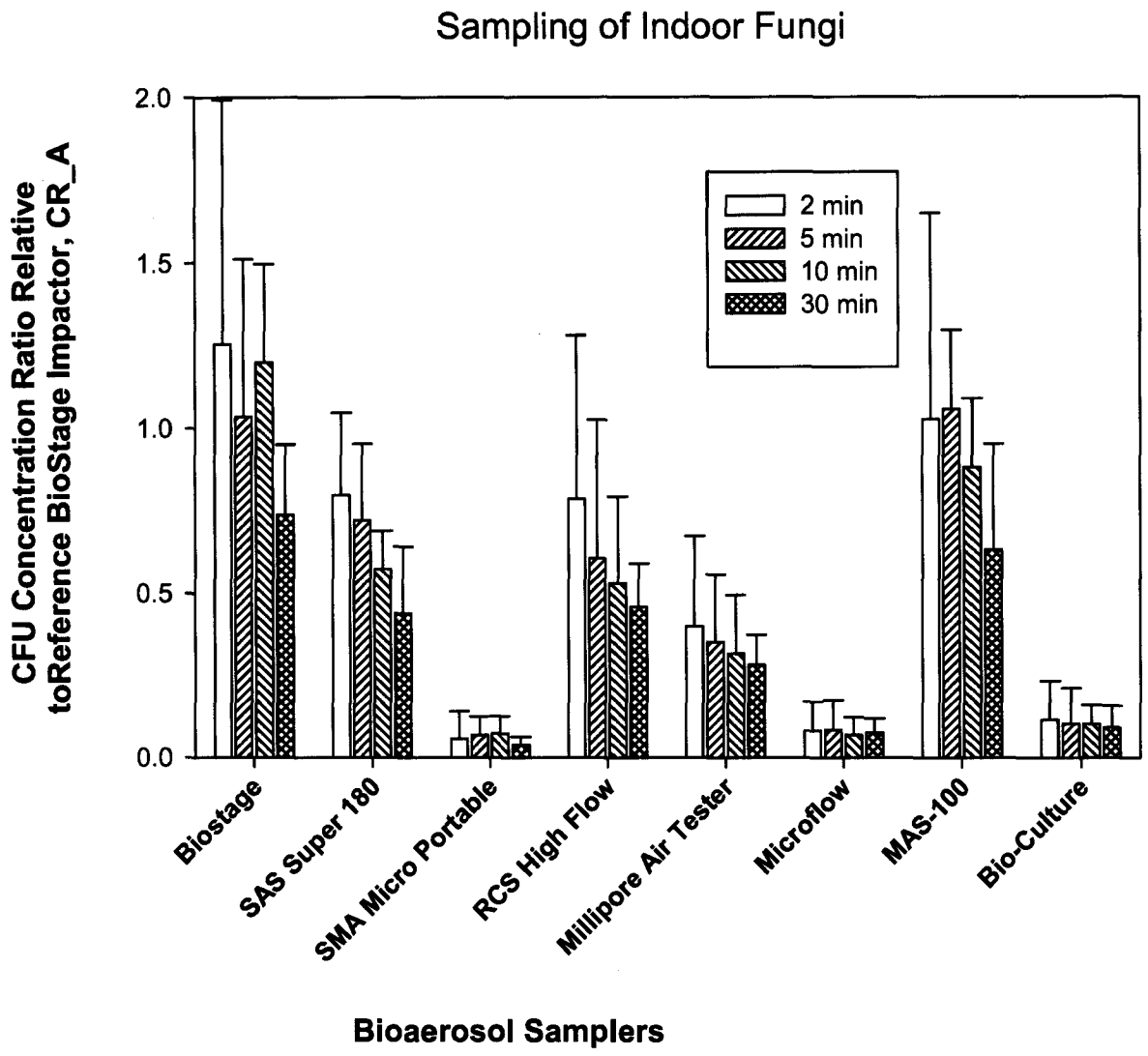

Figure 8.2. Relative performance of portable impactors as a function of sampling time, t_S, when sampling fungi indoors.

REF: Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology *to be submitted*.

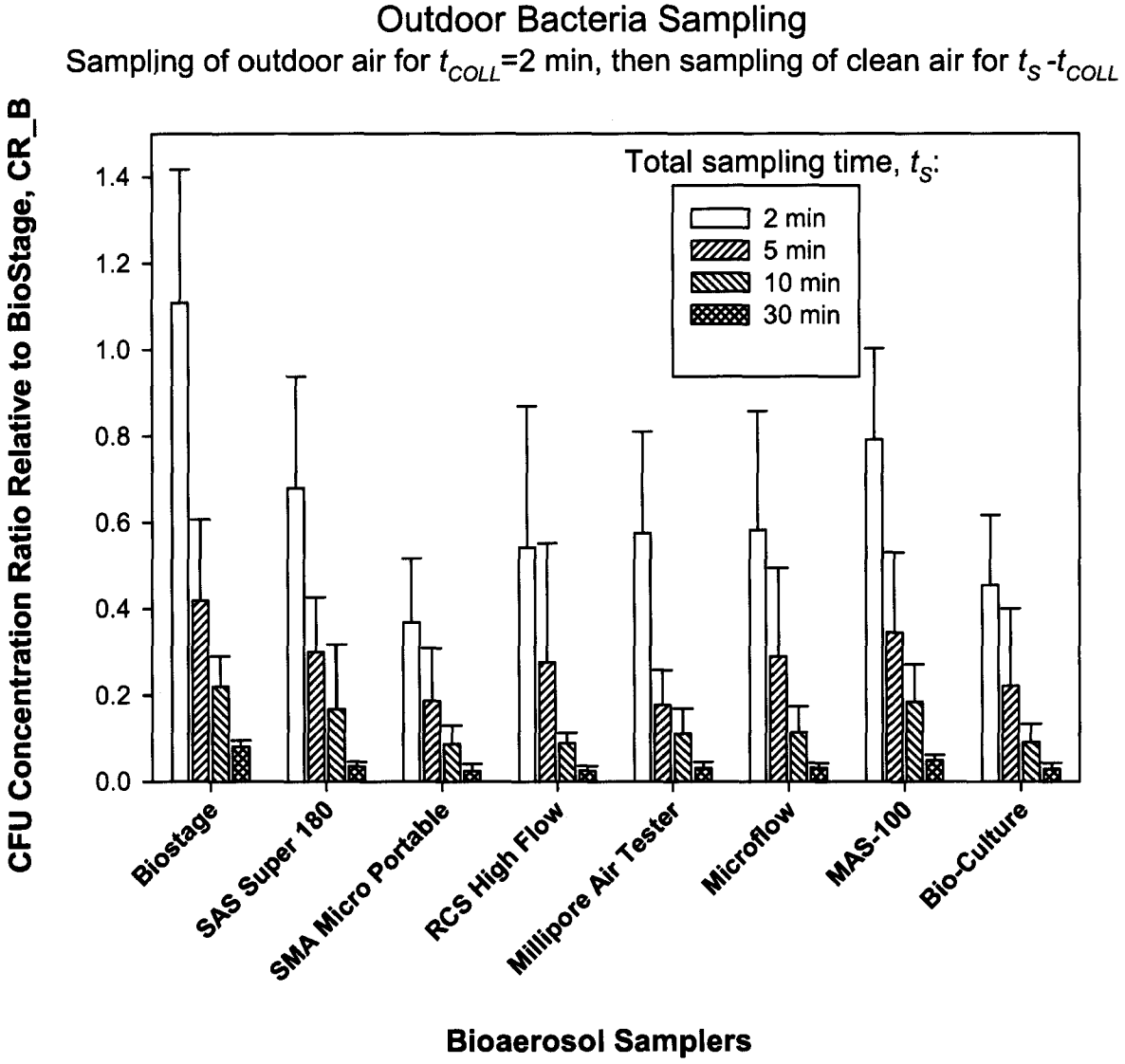

Figure 8.3. Relative performance of portable impactors as a function of sampling time, t_S, when sampling bacteria outdoors. The test samplers sampled outdoor air for t_{COLL}=2 min and then sampled particle-free air for t_S -t_{COLL}, where t_S = 2, 5, 10, and 30 minutes.

REF: Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology *to be submitted*.

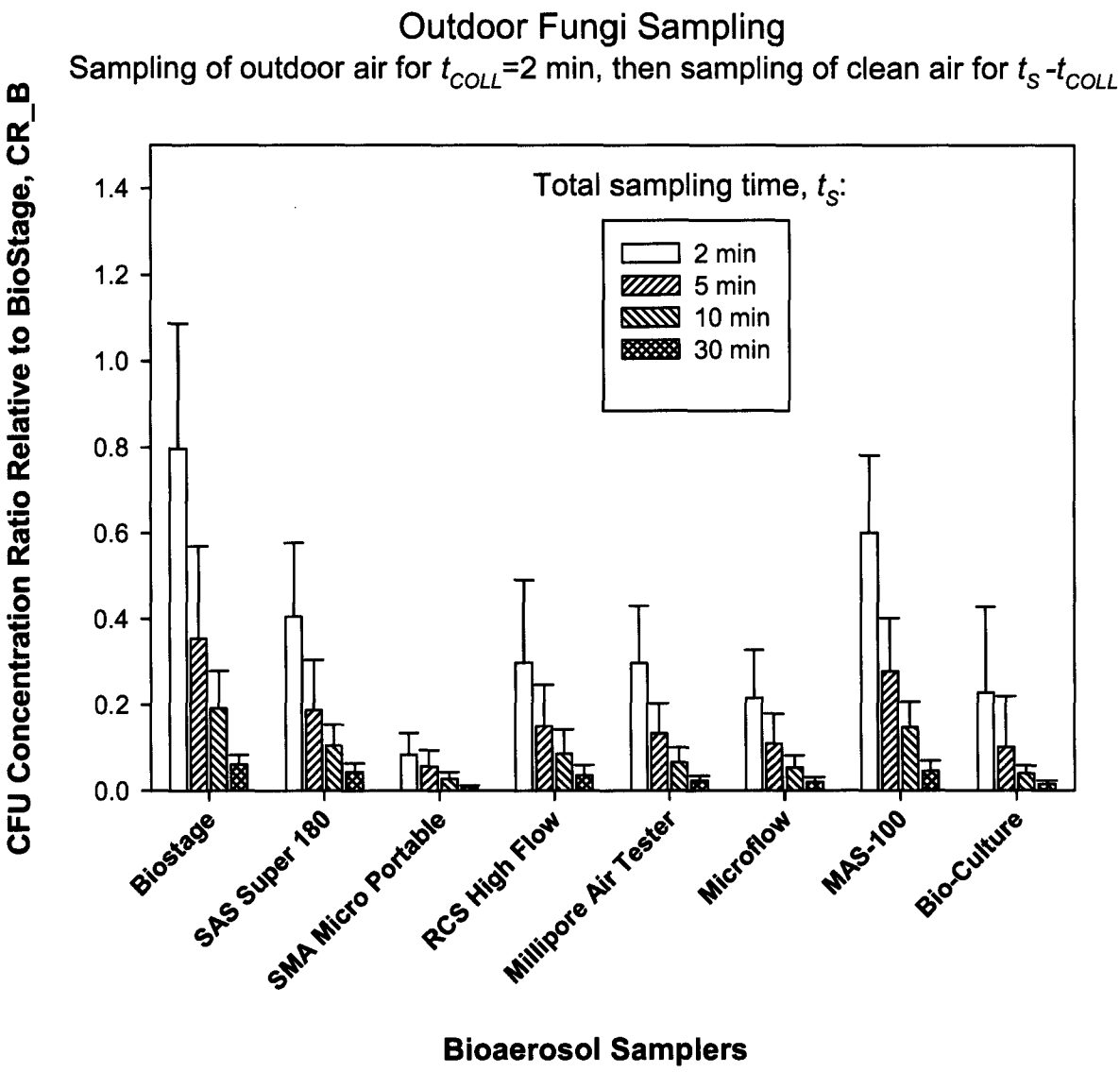

Figure 8.4. Relative performance of portable impactors as a function of sampling time, t_S, when sampling fungi outdoors. The test samplers sampled outdoor air for t_{COLL}=2 min and then sampled particle-free air for t_S -t_{COLL}, where t_S = 2, 5, 10, and 30 minutes.

REF: Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology *to be submitted.*

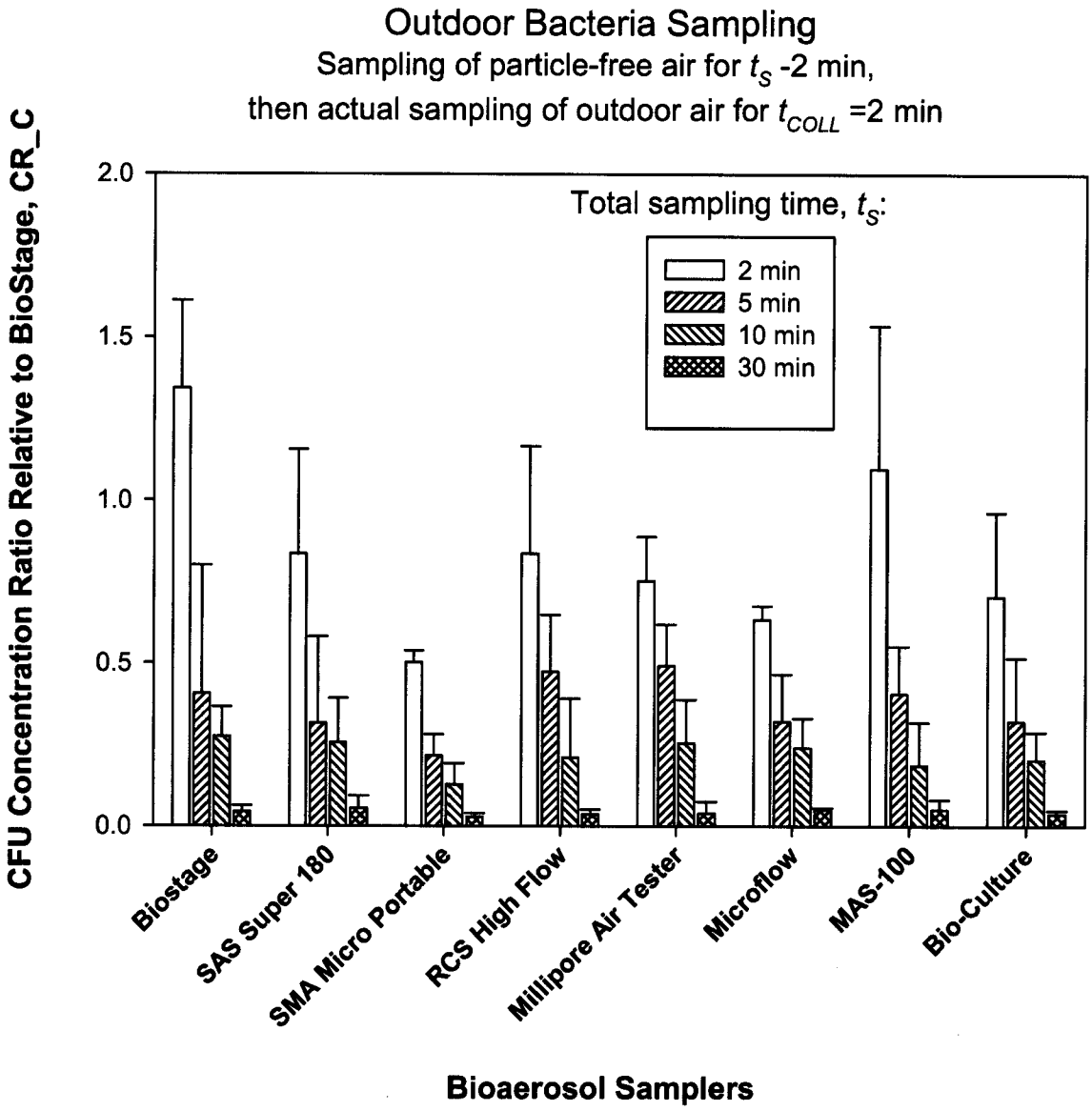

Figure 8.5. Relative performance of portable impactors as a function of sampling time, t_S, when sampling bacteria outdoors. The test samplers sampled particle-free air for t_S – 2 min (t_S = 2, 5, 10, and 30 minutes) and then sampled outdoor air for t_{COLL}= 2 min.

REF: Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology *to be submitted.*

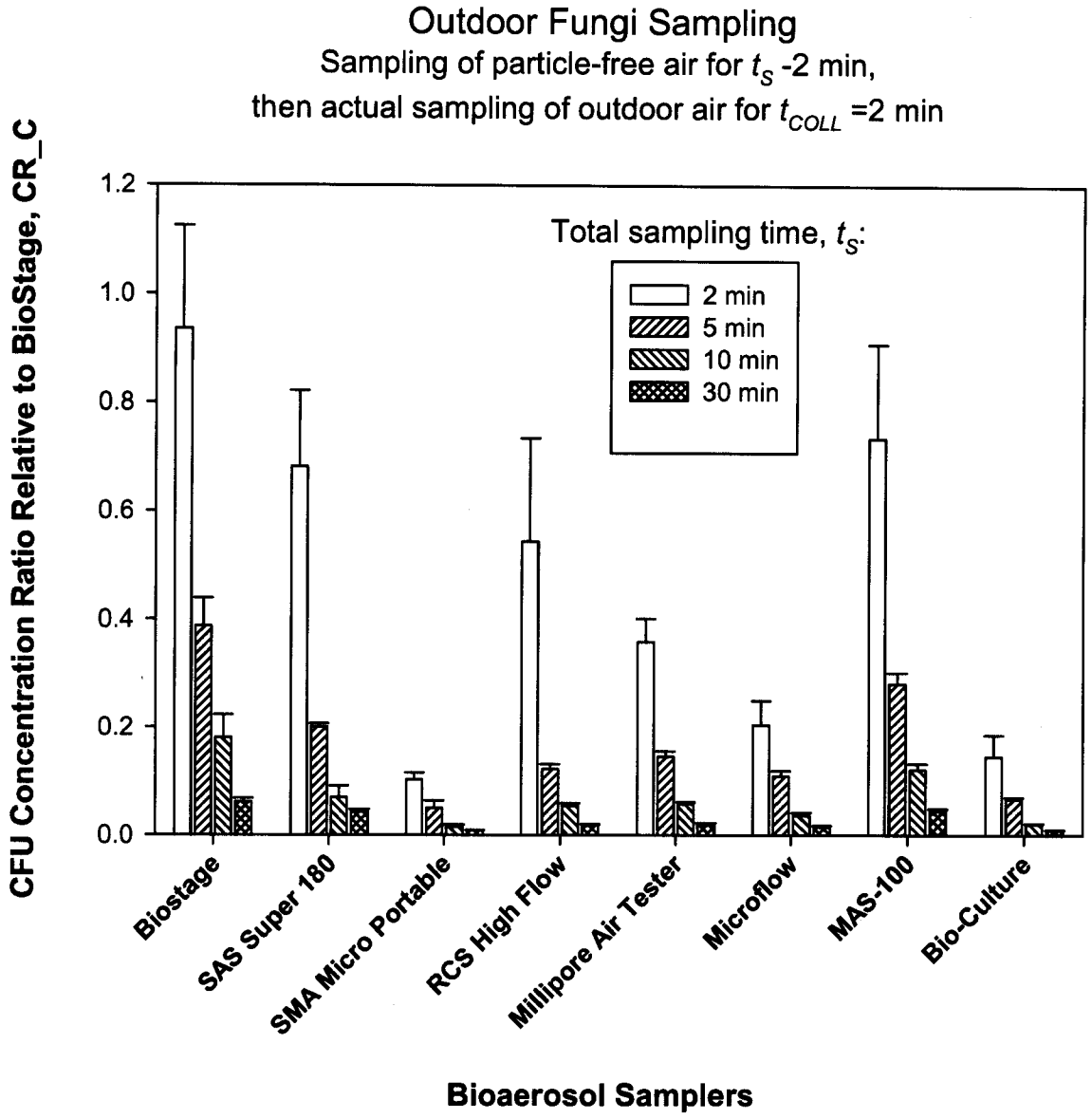

Figure 8.6. Relative performance of portable impactors as a function of sampling time, t_S, when sampling fungi outdoors. The test samplers sampled particle-free air for t_S – 2 min (t_S = 2, 5, 10, and 30 minutes) and then sampled outdoor air for t_{COLL}= 2 min.

REF: Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology *to be submitted.*

9 Specific Aim VIII: *Summary of the results*

In this research we analyzed the physical and biological performance of newly available or modernized portable bioaerosol samplers. The analyzed samplers included the MAS-100, Microflow, SMA MicroPortable, Millipore Air Tester, SAS Super 180, BioCulture, and RCS High Flow portable microbial samplers. Their performance was compared against a traditional reference bioaerosol sampler (BioStage impactor, which is Andersen-equivalent) and an inhalation-based sampler (Button Aerosol Sampler with gelatin filter) for viable bioaerosols. Through the theoretical analysis, laboratory and field experiments we determined whether these samplers can be used to accurately determine the levels of airborne viable bacteria and fungi. In addition, each sampler's physical performance was compared against the conventions for total inhalable particles and particle deposition in the lung. The results and recommendations produced by this research were published in peer-reviewed publications and presented at national and international scientific conferences and workshops. The information on the samplers' performance is expected to help in selecting an effective sampling tool for analyzing and solving a particular occupational health problem related to bioaerosol exposure. Information about the portable samplers' performance relative to a standard sampler (Andersen-equivalent impactor) should also aid in assessing the accuracy and validity of obtained concentrations of viable airborne microorganisms. The text below summarizes the main findings of the study.

Physical collection efficiency of the portable samplers

Very often collection efficiency measurements directly compare particle concentrations upstream and downstream of a sampler without considering the particle losses inside the sampler. Here, we developed a new approach which tests collection efficiencies of the sampler with and without agar medium loaded. This method thus allows estimating the effective collection efficiency, i.e., the fraction of incoming particles deposited onto the agar collection medium only. In addition, we were able to determine the extent of losses inside the samplers. The evaluation of physical collection efficiencies was performed with polystyrene latex particles (PSL) ranging in size from 0.5 to 10 μm different sizes and six bacterial and fungal species ranging from 0.61 to 3.14 μm in aerodynamic diameter.

When testing with PSL particles, 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 Millipore Air Tester; 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.

When collecting bacteria, the RCS High Flow and the MAS-100 collected 20-30%, while other samplers collected less than 10% of these bio-particles. When sampling fungi, the RCS High Flow and the SAS Super 180 deposited 80-90% of airborne spores on agar – highest among investigated samplers. Other samplers showed collection efficiencies of 10-60%. Comparison of samplers' collection efficiencies with particle inhalation convention curves showed that RCS High Flow and SAS Super 180 could be used to assess inhalation exposure to particles larger than 2.5 μm, such as fungal spores. Performance of RCS High Flow sampler was also reflective of the particle lung deposition pattern when sampling both bacteria and fungi. MAS-100 and SAS Super 180 matched the total deposition curve fairly well when collecting bacterial and fungi species, respectively. For other

tested samplers we observed substantial discrepancies between their performances and particle deposition efficiencies in the lung.

The obtained physical collection efficiency data indicate that the use of most of the tested portable 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 bacterial agglomerates, larger fungal spores, or biological particles attached to larger particles.

In general, based on our experimental results it appears that most of the portable samplers would provide only qualitative data when collecting individual bacteria. In addition, none of the samplers could be used to estimate inhalation exposure of bacterial-sized particles. 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 new samplers should also be able to mimic health-relevant sampling conventions (either inhalation or particle deposition in the lung) which would provide more accurate information about the inhaled or deposited amount of culturable biological aerosols and thus contribute to building dose-response relationships relevant to biological aerosols.

Relative biological efficiency of the portable samplers

The relative biological efficiency of the samplers was determined using three bacterial and three fungal species which were aerosolized and collected by the samplers under controlled humidity, 40-45%. The investigated portable samplers had impaction velocity from 1 to 15 m/s and jet-to-plate distance from 1.7 to 6mm. The ability of portable impactors to recover culturable bioaerosols was compared to that of an Andersen-equivalent type impactor and a Button Aerosol Sampler equipped with gelatin filter and their relative overall performance was determined. In order to determine physical parameters governing the performance of portable impactors, their relative overall efficiency was correlated with their collection efficiency, jet velocity and jet-to-plate distance.

Experimental data indicated that performance of portable microbial samplers varied from model to model, and depended on investigated bioaerosol species. When enumerating single bacterial cells, the BioStage impactor performed better than portable impactors and the relative overall performance of the impactors was highly correlated with their collection efficiency (adjusted R^2=0.74 - 0.94) and inclusion of samplers' jet velocity and jet-to-plate distance into multiple liner regression did not affect the adjusted R^2 values.

When enumerating single airborne fungal spores, the BioStage reference impactor also performed better than portable impactors, except for *C. cladosporioides*, when MAS-100 and SAS Super 180 performed equally well. Use of the collection efficiency in the linear regression as the sole predictor of the impactors' relative overall performance yielded adjusted R^2 values ranging from 0.61 to 0.73; inclusion of jet velocity and jet-to-plate distance into multiple liner regression increased the adjusted R^2 values to the range of 0.75 – 0.89. The data demonstrate that in addition to collection efficiency, jet velocity and jet-to-plate distance also play an important role in the enumeration of bioaerosols by microbial impactors.

Overall, among the investigated portable samplers, the MAS-100 impactor seemed to perform the best, while the RCS High Flow and the SAS Super 180 often had somewhat lower relative efficiencies. The

recovery of single bacteria and fungal spores by other impactors was substantially lower compared to the BioStage impactor. One has to note that this research analyzed impactor efficiency relative to the BioStage. If the BioStage has a low biological performance when sampling sensitive bacteria, then even a high relative efficiency of a portable impactor does not indicate its high absolute biological performance.

When sampling using Button Aerosol Sampler with gelatin filter, the physical collection efficiency was approximately 100%. However, we observed zero recovery of *P. fluorescens* and *E. coli* bacteria and lower recovery of *C. cladosporioides* fungal spores compared to the BioStage. The recovery of *B. subtilis* bacteria, *A. versicolor* and *P. melinii* spores was not statistically different from that of the BioStage impactor. The gelatin filter was used with 5 minute sampling time so that enough microorganisms would be collected without increasing the aerosolization rate. The complete absence of bacterial colonies and lower recovery of *C. cladosporioides* after such a relatively short sampling time also indicates the susceptibility of these microorganisms to desiccation stress and indicates limitations of using gelatine filter as a collection medium for viable organisms.

The results obtained here are hoped to contribute towards understanding of the factors affecting microorganism enumeration by microbial impactors and could serve as a reference when selecting a portable sampler for environmental monitoring and when designing new microbial samplers for collection of culturable bioaerosols.

Performance of samplers in a field environment

The performance of portable microbial samplers was investigated in indoor and outdoor environments when collecting bacteria and fungi and their performance was compared against that of a BioStage impactor and a Button Aerosol Sampler equipped with gelatin filter. The results showed the sampling environment can have a statistically significant effect on sampler performance, most likely due to the differences in airborne microorganism composition and/or their size distribution. Analysis of data polled from all trials using ANOVA showed that relative performance of all samplers was statistically different (lower) compared to the BioStage, except the RCS High Flow and the MAS-100. The MAS-100 also had statistically higher performance compared to other portable samplers, except the RCS High Flow. The Millipore Air Tester and the SMA had the lowest performances among the investigated samplers. The recovery of microorganisms on a gelatin filter was statistically significant (lower) compared to the BioStage impactor. On average, the microorganism concentration recovered on a gelatin filter was approximately 50% of that recovered by a BioStage impactor. The relative performance of the investigated impactors was successfully described using a multiple linear regression model ($R^2 = 0.83$) and the effects of samplers' cutoff sizes and jet-to-plate distances as predictor variables were statistically significant.

The data from this part of the study will help field professionals in selecting bioaerosol samplers for their needs. The developed empirical formula describing the overall performance of bioaerosol impactors can assist in sampler design.

This study has shown that different portable microbial samplers yielded different airborne microorganism concentrations in indoor and outdoor environments. It was also found that the impactor performance in a particular environment could vary from day to day, likely due to the different airborne microorganism composition and/or size distribution. Since the overall profile of the portable

samplers' performance remained consistent in different environments and over separate sampling days, the data presented in this study are expected to help field professionals and bioaerosol researchers in selecting bioaerosol samplers for their needs. The developed empirical formula to predict overall sampler performance might aid in designing more efficient bioaerosol samplers.

Effect of sampling time on performance of portable impactors

We analyzed whether the sampling time affects the overall performance of seven portable impactors mentioned above when collecting airborne bacteria and fungi from 2 to 30 min indoors and outdoors. In addition, to better ascertain the factors responsible to likely decrease in samplers' performance with prolonged sampling time, we separately investigated the effects of desiccation damage to the already collected microorganisms as well as the effect of agar desiccation prior to collecting the microbial particles.

The obtained data in our study clearly indicate that recovery of airborne microorganisms collected using portable impactors as well as traditional stationary impactors decreases as the sampling time increases. Once the organisms are deposited onto the collection media they are being exposed to air flow over them as the sampling continues causing their dehydration and inactivation. If an area under the impaction nozzle is exposed to air prior to depositing the organism it becomes desiccated and hard resulting in a microorganism bounce or reduced access to nutrients. The effect of both of these factors was statistically significant. The results of our investigation suggest that when impactors are used for the collection of airborne bacteria and fungi, sampling times should be reasonably short to minimize under-representation of airborne microorganism concentration. Otherwise, use of longer sampling times would result in reduced recovery of collected microorganisms which would under-report and misrepresent concentration of microorganisms in the air. Inaccurate data on airborne microorganism concentrations are likely to lead to erroneous conclusions of the investigation.

Publications

Journal articles

1. Yao M and Mainelis G: [2006] Investigation of Cutoff Sizes and Collection Efficiencies of Portable Microbial Samplers. Aerosol Science and Technology 40(8): 595-606.

This publication responds to Specific Aim I: "Development of the experimental system for testing the physical and biological efficiencies of portable bioaerosol samplers", Specific Aim II: "Theoretical analysis of test bioaerosol samplers", and Specific Aim III "Determination of the physical collection efficiency of the portable samplers when challenged with non-biological and biological test particles".

2. Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

This publication responds to Specific Aim III "Determination of the physical collection efficiency of the portable samplers when challenged with non-biological and biological test particles".

3. Yao M and Mainelis G: [2006] Effect of Physical and Biological Parameters on Enumeration of Bioaerosols by Portable Microbial Impactors. Journal of Aerosol Science 37(11): 1467-1483.

This publication responds to Specific Aim IV "Evaluation of physical and biological efficiency of the inhalable reference sampler for viable bioaerosols" and Specific Aim V "Evaluation of the biological performance of the portable bioaerosol samplers".

4. Yao M and Mainelis G: [2007] Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols. Journal of Occupational and Environmental Hygiene 4: 514-524.

This publication responds to Specific Aim VI "Field evaluation of the portable bioaerosol samplers"

5. Mainelis G and Tabayoyong M [2008] Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Aerosol Science and Technology to be submitted.

This publication responds to Specific Aim VII "Investigation of the sampling time effect on the performance of the samplers"

Proceedings

1. Mainelis G and Tabayoyong M: [2007 Effect of Sampling Time on the Overall Performance of Portable Microbial Impactors. Abstracts of the European Aerosol Conference 2007, Salzburg, Austria, September 9-14, 2007.
2. Grinshpun SA, Adhikari A, Cho SH, Reponen T, Mainelis G. and Yao M: [2007] Factors affecting the performance of bioaerosol impactors, Abstracts of the European Aerosol Conference 2007, Salzburg, Austria, September 9-14, 2007.
3. Mainelis G and Yao M: [2007] Field Performance of Portable Impactor when Enumerating Viable Bioaerosols, Abstracts of the American Industrial Hygiene Conference and Exposition, Philadelphia, Pensylvania, June 2-7, 2007.
4. Mainelis G and Yao M: [2006] Biological Performance of Portable Impactors When Collecting Airborne Bacteria and Fungi, Abstracts of the 7th International Aerosol Conference, St. Paul, Minnesota, September 10-15, 2006.
5. Mainelis G and Yao M: [2006] Performance of Portable Microbial Samplers When Collecting Inhalable Particles, Abstracts of the American Industrial Hygiene Conference and Exposition, Chicago, Illinois, May 13-18, 2006.
6. Yao M and Mainelis G: [2006] Performance of Portable Microbial Samplers for Estimating Human Exposure to Airborne Biological Agents, Abstracts of the National Occupational Research Agenda (NORA) Symposium 2006, Washington, DC, April 18-20, 2006.
7. Yao M and Mainelis G: [2005] Investigation of Collection Efficiencies and Inhalation Convention Conformity of Portable Microbial Samplers, Abstracts of the 24th Annual Meeting of the American Association for Aerosol Research, Austin, Texas, October 17-21, 2005.

Thesis

Yao M [2006] Sampling and Inactivation of Microorganisms in Addressing Human Exposure to Biological Agents, Ph. D. Thesis, Rutgers, The State University of New Jersey.

Inclusion of gender and minority study subjects

NA

Inclusion of children

NA

Materials available for other investigators

Experimental collection efficiency of an aerosol sampler is often determined by directly comparing particle concentrations upstream and downstream of the sampler without considering the particle losses inside it. Here, we developed a new approach which tests collection efficiencies of the samplers with and without agar medium loaded. This method thus allows estimating the effective collection efficiency, i.e., the fraction of incoming particles deposited onto the agar collection medium only and the extent of losses inside the samplers. This method is described in the following publication:

Yao M and Mainelis G: [2007] Use of Portable Microbial Samplers for Estimating Inhalation Exposure to Viable Biological Agents. Journal of Exposure Science and Environmental Epidemiology 17: 31-38.

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