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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. To improve exposure assessment and control, and to protect the populations and resources potentially exposed to airborne microbial agents, we evaluated a feasibility of a novel bioaerosol sampler capable of achieving very high sample concentration rates. High sample concentration rates allow detecting low microorganism concentrations thus improving our understanding of a relationship between biological exposures and health outcomes.

Airborne particles drawn into the sampler are electrostatically deposited onto a narrow collection electrode covered with superhydrophobic substance. The chamber is positioned at a small angle to the horizontal and a small liquid droplet introduced at the top of the collection chamber rolls down under gravity and collects the deposited particles. Sampler's performance was analyzed with non-biological and biological particles, collecting droplet volumes from 5 to 60 μL, and sampling flow rates from 2 to 10 L/min. It was found that the vast majority of particles deposited onto the electrode are removed by the first rolling droplet. When testing with polystyrene latex particles of 3.2 μm and the collecting droplet of 5 μL we achieved an unprecedented sample concentration rate as high as $1.2x10^6$. These concentration rates were sustained for sampling times as long as 60 min.

Experiments with the two common test bacteria (*Pseudomonas fluorescens* and *Bacillus subtilis*) have shown that the novel bioaerosol sampler can also efficiently collect and concentrate airborne bacteria in small amounts of liquid (5 or 40 μL). For 10 min sampling, the collection efficiencies for both bacteria ranged from 50 to 72% and were substantially higher compared to the collection efficiencies for PSL particles of similar size. When used with 5 μL collection droplet and 10 L/min sampling flow rate, the EPSS achieved sample concentration rates of $1.2x10^6$/min which are higher than those achieved by current bioaerosol samplers.

The sampler's performance was also analyzed with three common fungi (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) and it achieved concentration rates ranging from $1x10^5$ to $3x10^5$/min when tested with airborne spore concentrations of 10^2-10^3/L (10^5-10^6/m^3). For fungal concentrations commonly encountered in the ambient environment (10^3-10^4/m^3), the concentration rate of the EPSS approached 10^6/min and higher. In addition, we successfully developed and applied an ATP-based bioluminescence method to quantify the collected fungal spores. The spore concentrations determined by microscopy and the ATP-based method were not statistically different. In fact, the ATP-based method allowed us to analyze spore concentrations that were too low to reliably detect by microscopy.

The development of the new bioaerosol sampling concept introduces a new and efficient tool for determining exposure to airborne microorganisms in residential, occupational and environmental settings, thus improving our ability to protect populations at risk. The use of small collection liquid quantities makes the sampler compatible with various "laboratories on a chip" and may lead to near real-time determination of airborne microbial contaminants. Future studies will design a new particle charging unit, will develop field-deployable sampler and will test it exhaustively in various laboratory and field environments. Upon successful testing, the new sampler could be deployed in various occupational environments.

Highlights/Significant Findings

The main objective of this exploratory research was to improve our ability to measure exposures to low concentrations of airborne microorganisms by evaluating feasibility of a novel sampler with very high sample concentration rate. The prototype Electrostatic Precipitator with Superhydrophobic Surface (EPSS) has a shape of a closed half-pipe, where a top plate serves as the ground electrode, while the collecting surface is a 2-3mm wide rectangular electrode coated with a superhydrophobic substance and positioned in a groove in the flat bottom surface opposite the ground electrode. Airborne particles drawn into the sampler were positively charged and then by the action of an electrostatic field deposited onto the negatively charged electrode. The sampler was positioned at a ~5 degree inclination angle to the horizontal, and the water droplets injected at the top of the collection chamber rolled-off of electrode's surface removing deposited particles. Sampler's performance was initially analyzed with polystyrene latex particles of different aerodynamic diameters, collecting droplet volumes 5 to 60 μL, and sampling flow rates from 2 to 10 L/min. It was found that the vast majority of particles deposited onto the electrode are removed by the first rolling droplet. When testing with the collection electrode of 2.1 mm and lowering the droplet volume to 5 μL we achieved an unprecedented concentration rate as high as $1.2x10^6$. These concentration rates were sustained for sampling times as long as 60 min.

Experiments with the two common test bacteria (*Pseudomonas fluorescens* and *Bacillus subtilis*) have shown that the novel bioaerosol sampler can efficiently collect and concentrate airborne bacteria in small amounts of liquid (5 or 40 μL). For 10 min sampling, the collection efficiencies for both bacteria ranged from 50 to 72% and were substantially higher compared to the collection efficiencies for PSL particles of similar size.

As a part of our effort to improve the ability to quantify bacteria in samples, we investigated application of the whole-cell QPCR. In this method, DNA is not extracted extraction prior to the PCR reaction, but the whole cells are used as the reaction template. We developed calibration curves for this method and applied it to analyze performance of the EPSS. The collection efficiency of the EPSS determined by the traditional epifluorescence microscopy and the whole-cell QPCR was not statistically different for the two bacteria indicated above. When used with 5 μL collection droplet and 10 L/min sampling flow rate, the EPSS achieved sample concentration rates of $1.2x10^6$/min which are much higher than those achieved by current bioaerosol samplers.

The sampler's performance was also analyzed with three common fungi (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) and it achieved concentration rates in the range of $1x10^5$ to $3x10^5$/min when tested with airborne spore concentrations of 10^2-10^3/L. For fungal concentrations commonly encountered in the ambient environment (10^0-10^1/L), the concentration rate of the EPSS approached 10^6/min and higher. In addition, we successfully developed and applied an ATP-based bioluminescence method to quantify the collected fungal spores. The spore concentrations determined by the microscopy and the ATP-based method were not statistically different. In fact, the ATP-based method is faster and allowed us to analyze spore concentrations that were too low to reliable detect by microscopy.

Future studies will develop a field-deployable sampler and will test it exhaustively in the laboratory and field environments. Upon successful testing the new sampler could be deployed in various occupational environments.

Translation of Findings

Exposure to airborne biological agents is known to result in a high number of respiratory infection episodes and carries a heavy price tag in medical care cost and loss of income. The main objective of this exploratory research was to improve our ability to measure exposures to airborne microorganisms, especially to their low concentrations, by evaluating feasibility of a novel bioaerosol sampler. The proposed sampling method, where electrostatic collection technique is combined with the use of superhydrophobic collection surface, was proven to be feasible and the bench top model achieved unprecedented sample concentration rates of approximately 10^6/min when sampling airborne bacteria and fungi. Such concentration rates are ~ 2 orders of magnitude higher than those achieved by current bioaerosol samplers and would allow detection of low microorganism concentrations in residential, occupational and environmental settings, thus improving our ability to protect populations are risk.

This project only demonstrated the feasibility of the proposed bioaerosol collection method and built a bench top sampler model. In addition, it identified critical areas that need improvement, such as the development of an effective and reliable particle charging unit. The next step in this research would be to address those critical areas, build a field-deployable and portable sampler and exhaustively test it in laboratory and field environments. Upon successful testing, the new bioaerosol sampler would be deployed in various occupational environments to determine exposures to airborne biological agents.

Outcomes/Relevance/Impact

This exploratory research evaluated the feasibility of a novel bioaerosol sampling technique with very high sample concentration rate which is needed to improve our understanding of exposures thus allowing to better protect occupational populations at risk. The constructed bench top model has a shape of a closed half-pipe, where a top plate serves as the ground electrode, while the collecting surface is 3.2 mm wide rectangular electrode coated with a superhydrophobic substance and positioned in a groove in the flat bottom surface opposite to it. Airborne particles drawn into the sampler are positively charged and then by the action of an electrostatic field deposited onto the negatively charged electrode. The sampler is positioned at a ~5 degree inclination angle to the horizontal, so that the injected water droplets roll-off of electrode's surface removing deposited particles. The prototype sampler was characterized as achieving:

- Collection efficiency of ~50-70% and the resulting unprecedented concentration rate of $1.2x10^6$/min (!) when sampling bacteria at 10 L/min with 5 μL droplet.
- Concentration rate of up to $3x10^5$/min when sampling fungal spores at 10 L/min with 10 μL droplet. The concentration rate increases to ~10^6/min, when sampler is challenged with airborne fungal concentrations that are commonly found in ambient air (10^0-10^1/L).
- In most cases, a vast majority (>90%) of particles deposited on the collection electrode was removed by the first rolling droplet.
- Compatible with multiple sample analysis methods, including microscopy, quantitative whole-cell real-time PCR (QPCR) and adenosine triphosphate (ATP) – based luminescence. Both methods was developed and applied during this study.

The new sampling concept is feasible and upon its further development will provide a novel tool to estimate exposures to low bioaerosol concentrations. Further studies will develop and extensively test a portable and field-deployable sampler.

Scientific Report

1 Background

This grant application responded to PA-04-030 "NIOSH Exploratory/Developmental Grant Program (R21)". The exploratory research described here tested feasibility of a novel bioaerosol sampler capable of achieving very high sample concentration rate (1 million and higher) while featuring low power requirements. In this sampler, the airborne particles are electrostatically deposited on a superhydrophobic surface ("Lotus leaf" type) from where they are removed and collected by tiny (5 – 60 μL) rolling liquid droplets for subsequent analysis by microbiological techniques, such as culture-based, adenosine triphosphate (ATP)-based luminescence (developed as a part of this study) and quantitative real-time polymerase chain reaction (QPCR). A bioaerosol sampler with high particle concentration rate enables detection of low airborne microorganism concentrations. Low power consumption and small size would make such a sampler highly applicable for occupational and environment studies and field deployments. This research responded to several NORA's Priority Research Areas: Exposure Assessment Methods, Indoor Environment, Asthma and Infectious Diseases. The development and evaluation of new methods to assess exposure to workplace microbial contamination also responded to NIOSH's needs in Exposure Assessment Methods (NIOSH, 2002).

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 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 a 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 caused not only significant work disruption and economic loss, but, more importantly, posed fatal risks to present workers.

Bacterial infections, microbial allergy and toxicoses caused by exposure to bioaerosols in agriculture and in the food processing industry have been reported by Cox and Wathes (1995). It was found 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 various agricultural facilities (Millner, 2009); during silo uncapping (May et al., 1989) and unloading (Morey et al., 1989). Inhaling organic dusts containing fungal and actinomycete spores can induce allergic rhinitis, chronic bronchitis, extrinsic allergic alveolitis, and organic toxic dust syndrome (Vance and Weissfeld, 2007). Various illnesses and infections due to microbiological exposures have been found in metal working fluid environments (Kennedy et al., 1999; Kriebel et al., 1997; Robins et al., 1997), textile manufacturing industries (Schachter et al., 1984), solid waste treatment facilities (Chiba, 2009; Lembke and Kniseley, 1980; Rahkonen, 1990), food processing industries (Asefa et al., 2009; Tsai and Liu, 2009) and health-care environments (Kamboj and Sepkowitz, 2009). Adverse health effects such as asthma

and other allergic diseases due to biocontamination have been reported in occupational and residential indoor air environments (Burge, 1990; Fung and Hughson, 2008; Koskinen et al., 1995; Miller, 1992; Spengler et al., 1993). A significant portion of 'sick building syndrome' is thought to be associated with bioaerosol exposure (Douwes et al., 2008; Walinder et al., 2001). It was estimated that about 36% of residential homes in US have mold problems (Spengler et al., 1993). Concentrations of fungi and bacteria in schools were found to be sufficiently high to cause health problems (Daisey et al., 2003).

To improve exposure assessment and control, to locate sources of biological contamination, to analyze the transmission of infectious diseases and to protect the populations and resources potentially exposed to airborne microbial agents, advanced detection and sensing systems are needed for occupational health applications. Development of new tools for estimating our exposure to indoor and outdoor bioaerosols is especially important given the fact that perhaps 10% of urban and rural fine aerosol is biological in nature (Monn, 2001); however, our understanding of the bioaerosol presence in samples of fine particles is limited (Womiloju et al., 2003). As concluded in a review (Douwes et al., 2003), many bioaerosol species that may cause health effects are currently not identified and more research is needed to establish better tools to assess exposure to biological aerosols. In addition to improved exposure assessment, protection of the population and resources at risk from biowarfare agents requires advanced air sampling devices that enable detection of low agent concentrations.

Microorganisms in the air are often detected using the traditional plating and culturing technique (Herr et al., 2003) which quantifies the presence of biological agents that are viable and able to grow on a particular culture medium. Culture-based analytic methods can be very sensitive (even one viable cell may be detected), but the recovery of collected viable airborne microorganisms is often significantly decreased by the inactivation or loss during or after sampling (Buttner and Stetzenbach, 1993; Buttner et al., 1997). Thus, the determination of viable microorganism concentration in air samples is often not sufficient for exposure assessment purposes. In addition, a large fraction of airborne biological agents ($\geq$90-99%) is not culturable (DeLong and Pace, 2001). Also, the allergic response depends very often on the presence of specific cell components that are not necessarily directly related to the viability of microorganisms (Robbins, 2000; Speight et al., 1997). With the culturing technique, days and weeks are usually required for the microbial colonies to develop which prevents rapid decision making and can only tell us about exposures that have occurred days and weeks in the past.

Advanced sample analysis techniques, such as Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR), are highly sensitive, quantitative and much more rapid than culturing (Hensel and Petzoldt, 1995). Since these techniques detect the presence of not only viable but also non-viable microorganisms, they may avoid under-estimation of potentially allergenic microorganisms (Speight et al., 1997). While traditional PCR has limitations to quantify microbial presence (Birch, 2001), the quantitative real-time PCR (QPCR) method is capable of more reproducible, sensitive and accurate measurements of total microorganisms. This method been applied to estimate microorganism concentrations in environmental samples, including air samples (Kimura et al., 1999; Oppliger et al., 2008; Stetzenbach et al., 2004). Generally, when applying the QPCR to quantify environmental samples, one uses standard (or calibration) curves prepared beforehand to analyze environmental samples. The variables

affecting the accuracy of this method have been explored in our previous research (An et al., 2009; An et al., 2006). Application of the whole-cell QPCR, where entire cells are used as the PCR reaction template to quantify the collected bacteria, has been explored as a part of this project. In addition, for the detection of fungi we explored the application of ATP-based bioluminescence.

When bioaerosol samples are analyzed using QPCR and other modern analysis techniques, liquid is a preferred sampling medium. Filters are also used some times, but they require an additional preparation step: removing microorganisms from the filter into liquid. The commonly used liquid bioaerosol samplers are impingers and wetted-wall cyclones, such as AGI-4 and AGI-30 (Ace Glass, Inc., Vineland, NJ) or Multistage Liquid Impinger (Burkard Manufacturing Co. Ltd., Hertfordshire, UK), respectively. The BioSampler (SKC Inc., Eighty Four, PA) combines impingement and centrifugal forces in one sampler. These and similar samplers often feature satisfactory collection efficiencies and have found wide applications in residential and occupational bioaerosol exposure studies. Applicability of a liquid-based aerosol collector for exposure assessment is determined not only by its collection efficiency, but also by its sample concentration rate, i.e., rate with which particles present in an air volume are concentrated in a liquid volume per time period:

$$Concentration\ rate\ (t^{-1}) = \frac{Airborne\ particle\ concentration\ (L^{-1})}{Particle\ concentration\ in\ liquid\ (L^{-1})} = \frac{Q(L/\min)}{V(L)}\eta, \quad (1.1)$$

where Q is the sampling flow rate, V is the sample volume and η – collection efficiency. High concentration rates reduce the sampling time needed to detect airborne particles and enable detection of lower particle concentrations. Traditional liquid samplers operate at flow rates up to 20 L/min and feature low sample concentration rates, e.g. up to 2500 for BioSampler (SKC, Inc., Eighty Four, PA) operating at 12.5 L/min and sampling into 5 mL of liquid. Since the anthrax attacks of 2001, several new samplers have been developed for collecting airborne particles into liquid. Among those, InnovaTek, Inc. (Richland, WA) introduced the BioGuardian Air Sampler which operates from 100 to 1000 L/min and collects sample into 10-15 mL of liquid. The SpinCon air sampler by Specter Industries, Inc. (Kansas City, MO) samples at 450 L/min and concentrates sample into 10 mL of liquid. The BioCapture 650 (MesoSystems Technology, Inc., Albuquerque, NM) is a portable sampler that achieves a sampling flow rate of 200 L/min and collects particles into 2-5 mL of liquid. The concentration rates for these new samplers are in the order of tens of thousands. A new wetted-wall bioaerosol cyclone developed at the Texas A&M University has concentration rates of approximately has concentration rates in the range of $5x10^5$ - $6x10^5$ for 1 μm PSL particles. However, this cyclone features a continuous liquid outflow rate of 1 mL/min (Hu and McFarland, 2007; Seo, 2007). The Lawrence Livermore National Laboratory (Livermore, CA) has developed a stationary Autonomous Pathogen Detection System (APDS) that is capable of continuous and fully autonomous monitoring for multiple biowarfare organisms. The APDS operates at collection flow rates up to 3750 L/min and can achieve concentration rates as high as $7.5x10^5$/min when collecting 3 μm PSL particles into 4 mL of liquid (Mainelis et al., 2005). However, the size of the system, its power and cost requirements would make it difficult to apply in occupational and residential environments.

The need for easily deployable exposure assessment tools requires development of samplers with low power requirements. Compared to traditional inertia-based samplers, the electrostatic

collection technique has lower power requirements and still achieves efficient particle removal from the air. The PI of this proposal has successfully applied electrostatic precipitation for collection and enumeration of viable microorganisms (Mainelis et al., 2002a; Mainelis et al., 2002b; Yao and Mainelis, 2006b). A briefcase-sized electrostatic precipitator developed by the Savannah River Technology Center (Carlson et al., 2004) samples at air flow rate of 300 L/min and collects particles into 20 mL of liquid. An electrostatic sampler achieving similar collection flow rates was also presented by other authors (Coyle and Bindra, 2004). These samplers have maximum concentration rate of about 15 -20 thousands/min.

In our effort to advance exposure assessment tools, the goal of this exploratory research was to design and construct a prototype of an advanced bioaerosol sampler which would be compact, have low power requirements and, most importantly, would feature very high air sample concentration rate. In this sampler, the airborne microorganisms are electrostatically deposited on a superhydrophobic surface ("Lotus leaf" type) from which they are removed and collected by tiny (5 – 60 μL) rolling liquid droplets, e.g. a phenomenon known as self-cleaning. The liquid droplets containing the organisms are available for a subsequent analysis by QPCR and other techniques. Use of 10 L/min sampling flow rate in our prototype Electrostatic Precipitator with Superhydrophobic Surface (EPSS) could yield concentration up to 10^6/min. As other investigators have shown, electrostatic collectors can efficiently operate at sampling flow rates of 300 L/min (Carlson et al., 2004; Coyle and Bindra, 2004) which shows potential of the proposed technology to increase the concentration rates even higher.

Also, currently used liquid samplers, such cyclones and impingers, loose part of their collection fluid due to evaporation which limits their operation time (e.g., BioSampler, SKC, Inc.) or requires installation of complex and expensive liquid level maintenance systems (e.g., SASS 2000, Research International Inc., Monroe, WA). In our proposed EPSS sampler the sampling can proceed for a desired period of time and microliters of collection liquid have to be applied only after the sampling is complete.

One of the main components of the proposed sampling technology is the use of superhydrophobic (i.e. extremely unwettable) surface for particle deposition. The phenomenon of surface superhydrophobicity was noticed by observing the Lotus flower, which seemed to be able to stay free of dirt and pathogenic organisms. Microscopic examination of the flower revealed the presence of micro-structured surface as well as coating by water-repellent crystals. Due to such a structure, water contact angle is larger than 150 degrees which ensures that water droplet present on the surface rolls off at a slightest tilt, e.g. phenomenon is known as "Lotus-effect". In addition, adhesion between particles and rough and unwettable surfaces is reduced as well. Thus, when a water droplet rolls over a deposited dirt particle, the particle is wetted, adheres to the droplet and is removed from the surface as shown in Figure 1.1 Our preliminary experiments were performed with mincorTM S200 material (BASF GmbH, Ludwigshafen, Germany), which that simulates physicochemical properties of Lotus leaf and can be applied to other surfaces making them superhydrophobic. However, as we started the main set of testing, the manufacturer discontinued this product and alternative materials had to be found.

In summary, the goal of this project was to develop and test feasibility of an Electrostatic Precipitator with Superhydrophobic Surface (EPSS) with very high air sample concentration rate thus enabling detection and quantification of exposures to low airborne microorganism concentrations in various occupational and residential environments. The need for sensitive and specific methods for assessing exposure to bioaerosols and their mixtures is emphasized by NIOSH (2002). The same source stresses the need for non-culturing approaches. Utilization of the proposed collection method in a combination with QPCR and other techniques improves our understanding about exposures to mixtures of bioaerosol species of low concentrations. As such, the EPSS has a great potential for improving exposure assessment which would lead to improved protection of affected populations. Also, use of small liquid quantities makes the prototype sampler compatible with various "laboratories on a chip" and may lead to near real-time determination of airborne microbial contaminants.

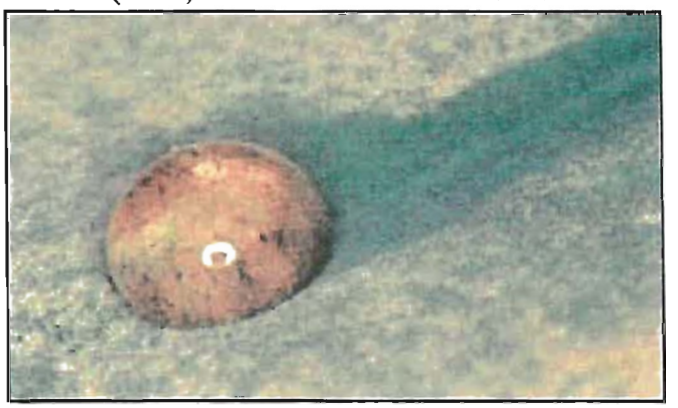

Figure 1.1 A rolling droplet picks up the particles covering a superhydrophobic surface thus cleaning it.

The following text describes design, construction and testing of the novel electrostatic sampler for biological particles. The tests were designed to investigate the feasibility of the proposed bioaerosol sampling concept and to investigate parameters affecting performance of an electrostatic sampler with superhydrophobic surface. At this research stage, majority of the experiments were performed in the laboratory.

2 *Objectives and specific aims*

The main objective of this exploratory research was to improve our ability to measure exposures to airborne microorganisms, especially to their low concentrations, by designing, constructing and evaluating a novel bioaerosol sampler. The proposed sampler was supposed to be compact, and feature very high air sample concentration rates (1 million and higher). In the proposed sampler concept, the airborne particles are electrostatically deposited on a superhydrophobic surface ("Lotus leaf" type) from which they are removed and collected by tiny (5–60 μL) rolling liquid droplets, e.g. a phenomenon known as self-cleaning. These liquid droplets containing collected microorganisms are then available for subsequent analysis by modern microbiological techniques, such as quantitative real-time polymerase chain reaction (QPCR). Thus, our underlying main hypotheses for the proposed research were that (i) combination of electrostatic collection method and use of superhydrophobic collection surface will allow achievement of sample concentration rates of 1 million and higher, (ii) the vast majority of particles deposited on the superhydrophobic surface would be picked up by 1 or 2 rolling droplets of 10-50 μL in volume, (iii) the proposed sampling concept would allow assessment of exposures even to low airborne microorganism concentrations.

The feasibility of the new sampling concept and its applicability for exposure assessment was evaluated in the laboratory using non-biological and biological (bacteria fungi) test particles. The overall performance (sample collection, extraction, and subsequent analysis) was evaluated against a reference bioaerosol sampler in the laboratory and field settings.

The research objectives were achieved and the hypotheses stated above were tested through the following Specific Aims:

I. Design and construction of Electrostatic Precipitator with Superhydrophobic Surface (EPSS).
II. Analysis of the sampler's performance when collecting non-biological particles.
III. Evaluation of the particle removal efficiency from the superhydrophobic surface as a function of droplet size and quantity.
    a. Analysis of the inclination angle effect on sampler's performance (ADDED AIM)
IV. Analysis of physical collection efficiency using biological test particles.
    a. Development and application of whole-cell QPCR techniques to quantify collected bacteria (ADDED AIM)
    b. Development and application of ATP-based bioluminescence technique to quantify collected fungi (ADDED AIM)
V. Laboratory evaluation of the EPSS against other bioaerosol samplers.
VI. Preliminary field evaluation of the EPSS against other bioaerosol samplers.
VII. Summary of the results, practical recommendations and future directions.

3 Specific Aim I: Design and construction of Electrostatic Precipitator with Superhydrophobic Surface (EPSS)

In the initial stage of our research, a sampler prototype was constructed based on the design described in the grant application and shown in Figure 3.1. The airborne particles drawn into the collector were imparted negative ions produced by an ionizer (Wein Products Inc., Los Angeles, CA). The negatively charged microorganisms then entered the collection chamber where by the action of electrostatic field they were deposited onto the ground electrode covered by superhydrophobic surface. The charging and precipitation chamber were inclined at angle θ (ca. 20 degrees) to the horizontal and had a form of a cylinder with diameter ca. 4 cm and length ca. 30 cm. The top half of the cylinder served as a high voltage (V_{EPSS}) electrode. The ground electrode was located opposite the high voltage electrode and was 3 mm wide. Due to such electrode configuration the electrical field lines were supposed to guide and deposit the charged particles onto superhydrophobic substance as shown in Figure 3.1b. Due to the "bulging" of the electrical field *E* lines at electrodes' edges, the collection chamber should have had little "dead space" where are no electrical field lines and thus, the vast majority of charged particles were supposed to be deposited on the superhydrophobic surface. A small fraction of the particles not collected by the device left the collector via exit. After the desired collection time, we injected liquid droplet(s) at the top of the collection chamber. Due to the inclination of the collection

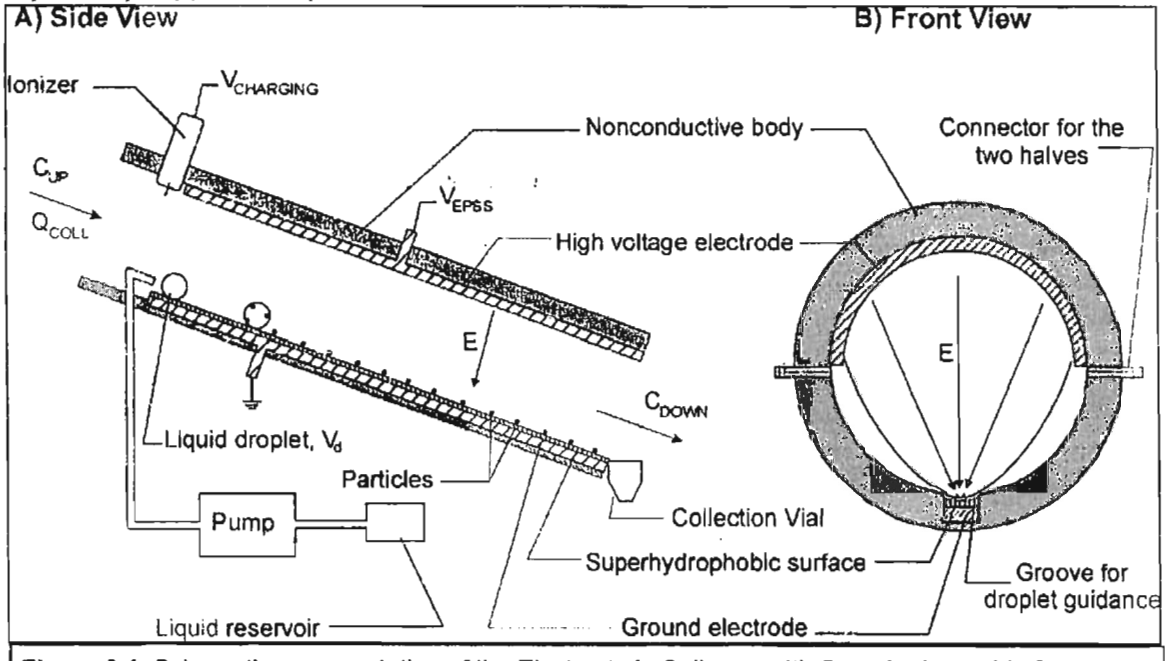

Figure 3.1. Schematic representation of the Electrostatic Collector with Superhydrophobic Surface (EPSS): A) Side view, B) Front view. The schematic is not to scale and is meant to show the principle of operation of the new device.

chamber and high liquid contact angle, the liquid droplets rolled down picking up the deposited particles and were deposited in a vial located at the end of the collection chamber. To make sure that the droplet rolls down the intended path (surface covered with collected particles) and not elsewhere, the ground electrode covered by the superhydrophobic substance was about 0.5 – 0.7 mm below the surrounding surfaces as shown Figure 3.1b.

The cylinder housing the charging and collection chambers was encapsulated by a nonconductive material and was built from two detachable halves (Figure 3.1b) so that they could be opened for cleaning and inspection. The ground electrode was also removable to allow better control of the electrode coverage by the superhydrophobic material. In addition, we needed the ability to remove the ground electrode to examine the particle deposition patterns and to determine the extent of possible losses.

The collection air flow Q_{COLL} through the new collector was provided by an external pump. Since the collector will be essentially an open channel device, there was very little pressure drop across it resulting in a low power consumption. The intensity of charging was regulated by adjusting the charging voltage $V_{CHARGING}$ (Figure 3.1a). The collection voltage, V_{EPSS}, was provided by a high voltage power supply (Bertan Associates, Inc, Valhalla, NY).

The preliminary experiments with polystyrene particles showed that particle retention inside the collector when operated at 10 L/min sampling flow rate, collection voltage of 9 kV and the charger at 50 mA current was marginal. Increase in the collection voltage was not feasible due to limitation of the power supply. The main reason for low particle collection was determined to be uneven distance from the high voltage electrode and the ground: the top of the high voltage electrode was at longer distance compared to its sides. In addition, the ionizer was found to induce substantial losses in the system, resulting in particle deposition on non-conductive surfaces of the sampler. Therefore, the sampler was redesigned as shown in Figure 3.2.

The device of the new design has a shape of a closed half cylinder and consists of 2 separate components: the round top part containing the ground electrode and the flat bottom part containing the collection electrode. Figure 3.2a presents a 3D view of the entire EPSS, while Figure 3.2b shows a 3D view of the bottom plate. The collection electrode is a thin copper strip coated with a superhydrophobic substance. When the collection electrode is placed inside the bottom plate, the top of the electrode is slightly below the surface of the plate forming a groove for improved guidance of the collecting droplet (Figure 3.2b). Two different collection electrode configurations were tested: the majority of the experiments were performed with an electrode 3.2 mm wide and positioned 0.5 mm below the plate surface. Some experiments were performed with an electrode that had the width of 2.1 mm and was 0.3 mm below the plate. In both configurations the length of the electrode was 254 mm. During the sampler's operation, the charged airborne particles are pulled into the sampler (charging conditions are described below) and the configuration of ground and collection electrode focuses the electrostatic field lines so that particles are subjected to an electrostatic field and deposited onto the collection electrode covered with the superhydrophobic substance. After a sampling period, liquid droplets of a desired volume are sequentially injected at the top of the collection plate using a micropipette. Due to the inclination of the collection chamber (1-30 degrees) and high liquid contact angle, the liquid droplets roll down picking up deposited particles and are collected in a vial located downstream of the collection chamber.

Although the collection electrode captures most of the particles, a fraction of the particles may be lost to the ground electrode and non-conductive parts of the sampler due to static charges. To minimize such losses, a large portion of the bottom plate is covered by an inlayed conductive

material (copper). The particles not captured by the EPSS are conveyed out of the device via exhaust.

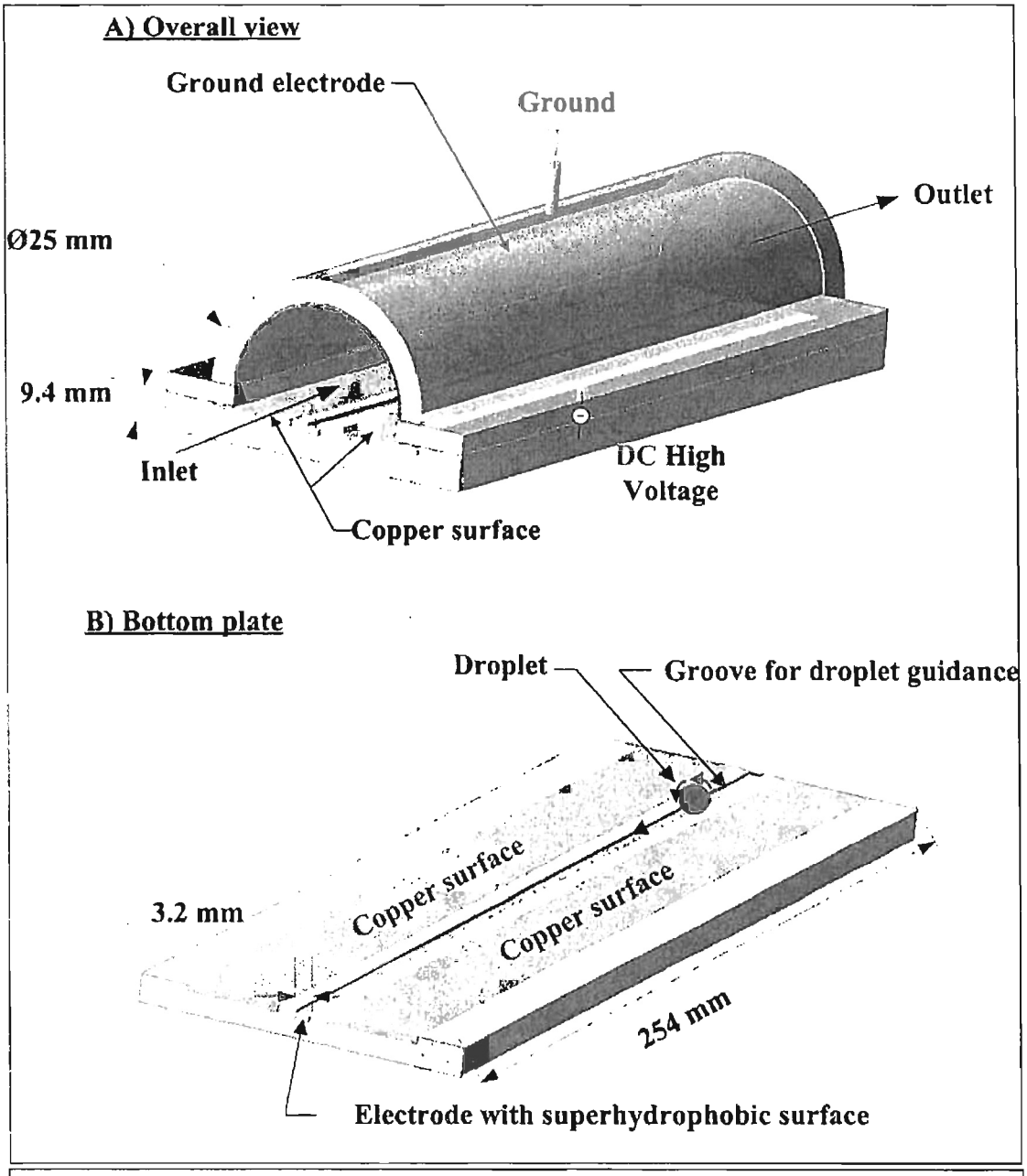

Figure 3.2. Schematic representation of the redesigned Electrostatic Collector with Superhydrophobic Surface (EPSS): A) Side view, B) Front view.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

4 *Analysis of sampler's performance when challenged with non-biological particles*

The experiments described here respond to Specific Aim II "Analysis of the sampler's performance when collecting non-biological polystyrene latex (PSL) particles" and Specific Aim III "Evaluation of the particle removal efficiency from the superhydrophobic surface as a function of droplet size and quantity". In addition, a set of experiments was performed to investigate the effect of inclination angle on the performance of the sampler (additional Specific Aim).

4.1 Experimental setup and testing methodology

Figure 4.1 shows the schematic of the experimental setup used to evaluate the performance of the electrostatic collector with superhydrophobic surface. The entire experimental setup was housed inside a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN) so that airborne particles not collected by the device are properly eliminated.

A Collison nebulizer (BGI Inc., Waltham, MA) was used to aerosolize the green fluorescent polystyrene latex (PSL) particles (Duke Scientific, Palo Alto, CA) from a liquid suspension at a flow rate, Q_A (4 L/min) and the test aerosol was dried and diluted with HEPA-filtered air flow, Q_D (36 L/min). The 40 L/min aerosol stream was passed through a 2-mCi Po-210 charge neutralizer to reduce aerosolization-related particle charges to Boltzmann equilibrium. The electrically neutralized particles then passed through a 0.035 m duct housing a vertically oriented ionizer (AS 150, Wein Products Inc., Los Angeles, CA) which imparted positive charge on the particles under controlled voltage and current settings. The positive charging of particles was selected because we found that this type of ionizer produces more stable concentration of airborne ions compared to negative ionizer. The electrically charged aerosol passed through a flow straightener and entered the testing chamber where it was collected by the EPSS at a flow rate Q_S.

During the tests, the EPSS was placed at a distance of 2-duct diameters downstream of the flow straightener and, depending on the test, collected particles from 10 to 60 min. A 47 mm glass fiber after-filter (Type A/E, Pall Inc., East Hills, NY) was used to collect the particles not deposited inside the EPSS, thereby allowing to determine the concentration of particles retained inside the EPSS.

In the preliminary experiments, collection electrode was covered by the superhydrophobic spray mincorTM S200 material (BASF GmbH, Ludwigshafen, Germany). Unfortunately, the company discontinued production of this material and we searched very hard to find a suitable replacement. Fortunately, we were able to find superhydrophobic spray HIREC-1450 (NTT Corporation Inc., Japan). All the experiments described below were performed with this material.

For each test, the collecting electrode was coated with superhydrophobic spray HIREC-1450 (NTT Corporation Inc., Japan) and left to dry at 60 °C for at least 1 hour. The coating procedure was repeated twice to achieve a uniform coating. One stable DC power supply (BK Precision,

Yorba Linda, CA) provided power to the ionizer (12V/50mA), while another stable DC high voltage power supply (Bertan Associates, Inc, Valhalla, NY) provided negative voltage (-7kV) to the precipitator to collect the positively charged particles. The operating values for these two voltages were established in the preliminary experiments as yielding the most efficient deposition of particles inside the EPSS and were not varied throughout the experiments. In future research projects, a new and more efficient charger will be designed and interfaced with the EPSS.

Fluorescent PSL particles of five sizes, i.e., 0.5, 1.2, 1.9, 3.2 and 5.1 μm in aerodynamic diameter (d_a) were used to characterize the sampler's performance at 3 collection flow rates, Q_S=2, 5, and 10 L/min, which were monitored using a mass flow meter (TSI Inc., Shoreview, MN). The particle sizes were selected to represent the most common size range of airborne bacteria and fungi.

After each test, the particles deposited on the superhydrophobic surface were removed by three sequential water droplets, which were collected in separate vials. The performance of the sampler was tested with water droplets of 5, 20, 40, and 60 μL. The mass concentration of PSL particles removed by each droplet, collected by the after-filter, as well as concentration of particles deposited on the ground electrode and elsewhere inside the sampler were quantified using a fluorometer (Sequoia-Turner Corp., Mountain View, CA).

Each sequential hydrosol sample (1st, 2nd, and 3rd water droplets) collected in a vial was evaporated using a heat gun (Master-Carr, Inc., Robbinsville, NJ) and then 4 mL ethyl acetate (EMD Chemicals Inc, Gibbstown, NJ) was added to the vial and set aside for 20 minutes to dissolve the PSL particles. An after-filter containing particles that escaped the EPSS sampler was soaked in 25 mL of ethyl acetate in a glass container for 4 hours to elute the fluorescein dye from the PSL particles. The mass of PSL particles remaining on the collection electrode (not removed by three rolling droplets) as well as the mass of PSL particles deposited on other components of the collector was quantified by extracting them using a defined quantity of ethyl acetate and analyzing using a fluorometer.

The concentration of aerosolized PSL particles was such as to ensure that fluorometer reading of each sample was approximately ten-fold of the background fluorescence of ethyl acetate and the measurements were adjusted for background readings. In addition, the concentrations of all analyzed samples were within the linearity range of the fluorometer.

A fraction of particles deposited in any individual part of the system (water droplet(s), collection electrode, ground electrode, bottom plate, or after-filter), η_i, can be defined as a ratio of the relative concentration of particles in the individual part, C_i, to the total relative concentration of the aerosol entering the system, C_{TOTAL}, and can be expressed as:

$$\eta_i = \frac{C_i}{C_{TOTAL}} = \frac{C_i}{\sum_i C_i} \tag{4.1}$$

The C_{TOTAL} is the sum of aerosol particle concentration in all individual parts of the system and is easily obtainable once the individual concentrations are determined. For all testing conditions the sampling was isoaxial and isokinetic or near-isokinetic and, according to formulas provided by

Hinds (1999), the inlet efficiency for 3.2 μm particles was 96% and greater. In addition, the C_{TOTAL} for 3.2 μm particles was compared against particle concentration in the test chamber (upstream of the sampler) measured by a filter in an isokinetic probe and the two concentrations were found to agree within 4%. Thus, to minimize the numbers of measurements, the C_{TOTAL} was used as a reference value for calculating various performance parameters of the sampler.

The relative aerosol concentration, C_i, for each part is calculated from:

$$C_i = \frac{I_i \cdot V_i}{Q_S \cdot t} \tag{4.2}$$

Here, I_i = concentration of fluorescein eluted in ethyl acetate (fluorometer reading) for a component i; V_i=volume of solution used to elute the tracer for a component i; Q_S= air sampling flow rate; and, t=sampling time.

The efficiency with which the particles deposited on the collection electrode were removed by each sequential water droplet, η_{Rj}, can be expressed as:

$$\eta_{Rj} = \frac{C_{WDj}}{C_{Electrode} + \sum_j C_{WDj}} = \frac{C_{WDj}}{C_{Electrode+WDs}} \tag{4.3}$$

where C_{WDj} is PSL particle concentration in each sequential water droplet (j = 1, 2, or 3), $C_{Electrode}$ is the concentration of particles remaining on the electrode (not removed by three water droplets) and $C_{Electrode+WDs}$ is concentration of particles deposited on the electrode during the sampling. The $C_{Electrode}$ was determined by washing the electrode with a defined amount of ethyl acetate and then determining the concentration of particles using Eq. 2. The concentration of particles in each water droplet, C_{WDj}, relative to the mass of all particles entering the sampler also determines the overall collection efficiency of the sampler based on the *j-th* water droplet, η_{Ej}:

$$\eta_{Ej} = \frac{C_{WDj}}{\sum_i C_i} \tag{4.4}$$

The η_{Ej} value incorporates the efficiency with which the particles are deposited on the collecting electrode and extracted from it into *j-th* water droplet.

In addition to the collection efficiency, another metric that describes the performance of an aerosol collector is the concentration rate, which represents the ratio of particle concentration in the liquid vs the concentration of particles in the air over a period of time. The concentration rate R_{Cj} with units of min^{-1}, based on the *j-th* droplet could be expressed as follows:

$$R_{Cj} = \frac{Q_S}{V_{WDj}} \times \eta_{Ej} \tag{4.5}$$

where V_{WDj} is the volume of the *j-th* liquid droplet.

4.1.1 Data Reproducibility

It is important to determine not only the particle deposition on the electrode and other components of the EPSS, but also the reproducibility of results from one set of experiments to

another, including the uncertainty (precision) of the results. For each set of repeated experiments one can determine a Coefficient of Variation (*COV*) as follows:

$$COV = \frac{\sqrt{\frac{1}{N}\sum_{k=1}^{N}(x_k - \bar{x})^2}}{\bar{x}}, \tag{4.6}$$

where N is the number of repeats; x_k is a value for a repeat k; and, $\bar{x}$ is value of the mean. When n sets of the same experiments are performed, one can calculate the mean value of the *COV*s, $\overline{COV}$, as well as the standard deviation of the *COV*s, σ_{cov}, from several sets of experiments as:

$$\overline{COV} = \frac{1}{n}\sum_{i=1}^{n} COV_i$$
$$\sigma_{COV} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(COV_i - \overline{COV})^2} \tag{4.7}$$

The inherant reproducibility of the measurement data could be expressed by calculating the Relative Precision, *RP*, which is defined as $RP = \sigma_{COV} / \overline{COV}$ (McFarland et al., 1999). The *RP* value indicates the ability of a particular experimental setup and methods to maintain precision from one set of experiments to another. The *RP* accounts for the variability in random error, but does not account for any systematic error and its changes from one set of experiments to another.

4.2 Results and discussion

Collection efficiency of electrostatic precipitators is often expressed as a function of charging voltage and current and/or collection voltage at different particle sizes or flow rates. However, since the primary focus of this project was to examine particle deposition and removal processes inside the EPSS as well as its ability to concentrate the collected particles in a small droplet, the current and voltage values were kept constant throughout the experiments.

Figure 4.2 shows the deposition fractions inside the EPSS for different PSL particle sizes (d_a=0.5, 1.2, and 3.2 μm) and, different water droplet sizes (10, 20, 40, and 60 μL) when sampling at a flow rate of 10 L/min and at ~5 degree inclination angle. In these figures, the η_i is the deposition fraction for each these components: cumulative for 3 water droplets, remaining on collecting electrode (particles not removed by the 3 droplets), ground electrode, bottom plate, and after-filter. It could be seen that for each particle size, the distribution of deposition fractions is similar across water droplet sizes, although larger droplets seem to accrue a larger fraction of deposited particles. The fraction of particles initially deposited on the collecting electrode (particles in water droplets plus those remaining on the electrode), $C_{Electrode+WDs}$, increased with increasing particle size. For each particle size, the $C_{Electrode+WDs}$ averaged over droplet sizes was approximately 58.5±4.1%, 72.5±3.8%, and 88.0±3.7%, respectively.

In Stokes regime, the electrical mobility of a charged particle is directly proportional to the amount of charge on the particle and inversely proportional to the particle diameter (Hinds, 1999). Since the amount of charge acquired by a particle in the field charging regime is proportional to d_p^2 (Hinds, 1999), the electrical mobility is directly proportional to the particle

diameter when charging conditions remain the same. Since the charging conditions were constant and the collection efficiency is a direct function of electrical mobility, this explains why higher particle fraction was deposited on the collection electrode as the particle diameter increased.

The EPSS uses electrostatic forces to remove charged particles from the aerosol stream onto the collection electrode, while uncharged particles or particles carrying low charge may be retained on other components of the EPSS (ground electrode or bottom surface) due to inertial or gravitational effects. Given sufficient amount of electrical charge on the airborne particles, deposition by electrostatic forces is expected to be the dominant mechanism. The extent of particle losses inside the EPSS (particles deposited on surfaces other than the collection electrode) decreased from 23-32% range for 0.5 μm particles to 4-9% range for 3.2 μm particles. The fraction of particles collected on the after-filter decreased from 13.7% for 0.5 μm particles to 5.4% for 3.2 μm particles. These results are again related to the electrical mobility of the particles.

The experiments conducted above indicated that the particles can be effectively deposited on the collection electrode. However, for a successful application of this sampling method it was important to determine what fraction of particles deposited on the superhydrophobic surface can be removed by each sequential rolling droplet. These data as a function of particle and droplet size are shown in Figure 4.3 for ~5 degree inclination angle of the EPSS. The most important result observed here is that the vast majority of particles that can be removed from the electrode is removed by the first rolling water droplet. The second droplet removed only a few percent of the deposited particles, while the third droplet removed 1-2% in most cases. This result is important because accumulation of most of the particles in a single droplet allows achieving higher concentration rates. If two droplets were needed, the concentration rate would be cut in half. It could also be seen that the removal efficiency, η_{Rj}, increases as a function of droplet volume, especially for the smallest, 0.5 μm PSL particles. For the 1.2 and 3.2 μm particles, the η_{Rj} of the first droplet is 40 and 65%, respectively, for the 10 μL droplet. For larger water droplets the removal efficiency increases to 53-57% for 1.2 μm particles and to 75-80% for 3.2 μm particles. This observation is most likely due to the fact that smaller particles are generally more difficult to remove from surfaces (Hinds, 1999).

Figure 4.4 shows the 3.2 μm PSL particle removal efficiency, η_{Rj}, by 40 μL water droplet from the superhydrophobic surface as a function of the inclination angle (1, 5, 10, 20, and 30 degree) at 10 L/min sampling flow rate. It could be seen from this Figure that the particle removal efficiency decreases for as the inclination angle increases to 20 degrees and higher. The average particle removal efficiency for the 1st water droplet at 1 to 10 degree angle was 78.3±0.8%, but it has decreased to about 65.3±2.0% for 20 degree angle and 56.9±2.9% for 30 degree angle. Since at higher angles the droplet tends to roll off quicker under the effect of gravity, the observed decrease is likely due to the shorter contact time of the droplet and the surface. Thus, to ensure a more efficient removal of particles deposited on the superhydrophobic surface of the new sampler, the inclination angle should be 10 degrees or less.

Since the earlier experiments indicated that the vast majority of collected particles are removed by the 1st water droplet, the overall collection efficiency and the concentration rate of the sampler

were also calculated (Equations 4.4 and 4.5) based on the values obtained with the 1st water droplet. The value of this overall collection efficiency, η_E, is a product of the efficiency with which the particles are deposited on the collecting electrode and the efficiency of particle extraction from the electrode by the 1st water droplet. The overall collection efficiency, η_E, and concentration rate, R_C, are presented in Figures 4.5 and 4.6, respectively, for two water droplet volumes (20 μL versus 40 μL) as a function of particle size (d_a = 0.5, 1.2, 1.9, 3.2, and 5.1 μm) and different sampling flow rates (2, 5, and 10 L/min). The particle charging and collection voltage settings were kept the same as in previous experiments. In these figures, the symbols indicate average values and standard deviations from triplicate measurements, while the solid lines are linear regression lines. As could be seen from Figure 4.5, the collection efficiency clearly increases for larger particles. This is a result of a more efficient removal of larger particles from the deposition electrode as was shown in Figure 4.3. Another result to note from Figure 4.5 is an increase in collection efficiency with increasing sampling flow rate. Although particle residence time inside the EPSS decreases at higher flowrates, our analysis of the deposition pattern inside the EPSS showed the reduction of losses inside the EPSS (particles on surfaces other than collection electrode) at increasing flow rates. At lower flow rates, gravitational settling may contribute to considerable losses which are minimized as the flow rate is increased (McFarland et al., 2002). In addition, any losses due to image charge effects are also reduced as the flow rate increases and residence time decreases.

About 90% of ambient bacterial aerosols are larger than 2.1 μm and more than 75% of such particles are larger than 3.3 μm (Lighthart, 1997). Based on Figure 4.5, the collection efficiencies using 20 μL and 40 μL water droplets for particles 3.2 μm and larger ranged from 42 to72% at 5 L/min and 47-77% at 10 L/min compared to 36-62% at 2 L/min. Using these collection efficiency data, we calculated the concentration rates for 20 μL and 40 μL droplet volumes and each sampling flow rate which are presented in Figure 4.6. As could be seen, for 20 μL droplet and the sampling flow rate of 10 L/min the concentration rate for 1.9 μm particles is 240,000 and reaches 382,000 for 5.1 μm particles. These values are much higher than concentration rates achieved by other compact samplers as summarized in the Introduction. When the droplet volume increases to 40 μL, the concentration rate averaged over all particles sizes decreases by approximately a factor of 1.7 compared to 20 μL droplet (Figure 4.6) mostly due to the dilution of the sample in larger droplet volume. On the other hand, reduction of the droplet volume to 10 μL, would increases the concentration rate to approximately 600,000.

Another factor affecting the concentration rate (R_C) may be the width of the collection electrode relative to the droplet size. Thus, to possibly improve the concentration rate even further by using a smaller droplet (5 μL), the width of the collection electrode was reduced by a third from its original size: from 3.2 mm to 2.1 mm. The width of the new electrode corresponds to the diameter of 5 μL water droplet which ensures that the droplet covers most of the width of the collection electrode. The results in Figure 4.7 achieved with 3.2 μm particles indicate that the concentration rate increases when a narrower electrode is used (data for 10 and 20 μL droplets). More importantly, a narrower electrode (2.1 mm) allows application of a tiny droplet of 5 μL with which an unprecedented concentration rate of 1.2 million is achieved when sampling 3.2 μm PSL particles at a flow rate of 10 L/min. Thus, the EPSS can efficiently operate at a sampling flow rate of 10 L/min with about 30-500 times higher concentration rate as compared to other liquid samplers (e.g., Biosampler, BioGuardian, BioCapture, etc.).

A number of current analytical methods use at least 50 μL of liquid for the sample analysis and may not yet benefit from the use of droplets as small as 5 μL. However, as analytical technology advances towards "laboratories-on-a-chip", a particle collector capable of concentrating particles in small amounts of liquid would be needed for successful integration of collection and detection capabilities.

All the data presented above were achieved with the sampling time of 10 min. In many bioaerosol sampling applications longer sampling times are needed. Figure 4.8a shows the fraction of 3.2 μm PSL particles deposited on the electrode, $C_{electrode+WDs}$, relative to all other components on the sampler, C_{TOTAL}. Figure 4.8b shows the collection efficiency, η_E, for sampling times of 10, 30 and 60 min. After 10 min sampling, about 80% of particles are deposited on the electrode, with the rest of the particles deposited elsewhere in the EPSS. As the sampling continues, the unipolarly charged particles deposited not on the collection electrode begin to repel each other, and the electrode potential becomes dominant force for particle collection and, thus the fraction of particles deposited on the electrode increases. After 30 and 60 minutes of sampling, the deposition on the electrode increased on an average by 14% and 16%, respectively, for all sampling flow rates. As far as the collection efficiency based on the 1st water droplet (Figure 4.8b), it increased with longer sampling time and exhibited trends similar to those observed in Figure 4.8a. However, after 60 minute of sampling the collection efficiency at 10 L/min decreased by 10% as compared to 30 minutes sampling time. Most likely because the 40 μL water droplet has become saturated and its ability to remove the particles deposited on the electrode after 60 min sampling has decreased. The decrease may not be observed in experiments with lower particle concentrations, such as encountered in ambient environment.

The main application of this sampling technology would be for collection of airborne microorganisms. Using the optimum sampling parameters determined in this research, we are currently experimenting with bacterial cells. The data indicate that the new sampling methodology allows achieving collection efficiencies of 50-60% and concentration rates as high 1.2 million.

4.3 Discussion of data reproducibility

The reproducibility of the data was examined using the methodology presented in "Testing Methodology" section and by performing two separate sets of tests in triplicate with 40 μL water droplet at 2, 5, and 10 L/min sampling flow rates with 3.2 μm PSL particles. The results are presented in Table 4.1. Charging conditions were kept constant at 12 V/50 mA for the charging ionizer and the sampling was performed with 7 kV sampling voltage. For each set of tests, we calculated a *COV* for each particle deposition fraction (particles removed by the 1st, 2nd, and 3rd water droplets, remaining on the electrode or other surfaces, and collected on the after-filter), average *COV* as well as the relative precision.

As could be seen from Table 4.1, the value of the relative precision averaged for all deposition fractions was $RP = 0.36$. This value represents the inherent reproducibility of our measurement methodology. The achieved *RP* value of 0.36 implies that for a mean *COV* of 10% the standard

deviation among *COVs* would be 3.6%; for the *COV* of 20%, the standard deviation would be 7.2%. These values indicate that our experiments were highly repeatable.

4.4 Figures and Tables for Chapter 4

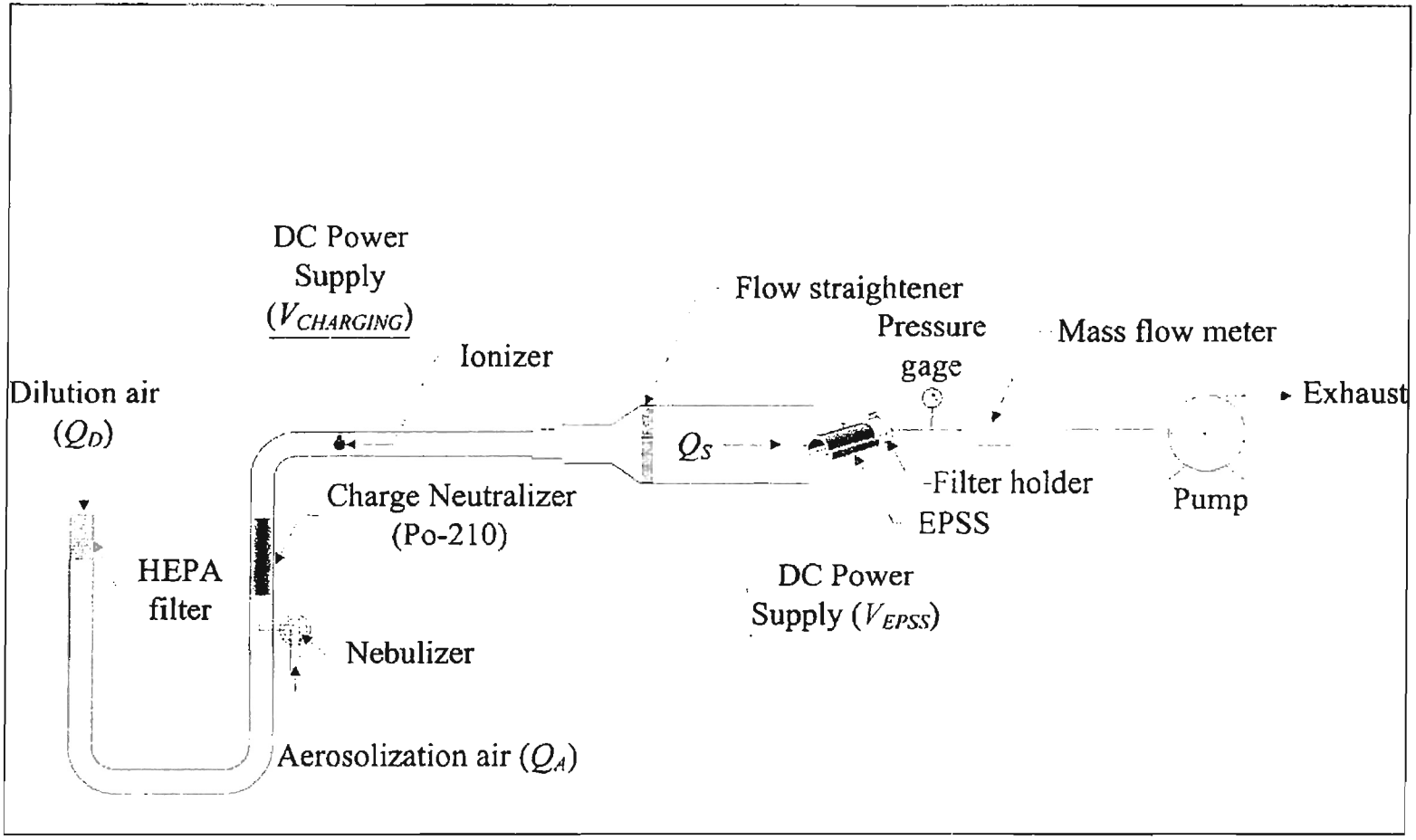

Figure 4.1. The schematic diagram of experimental setup

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

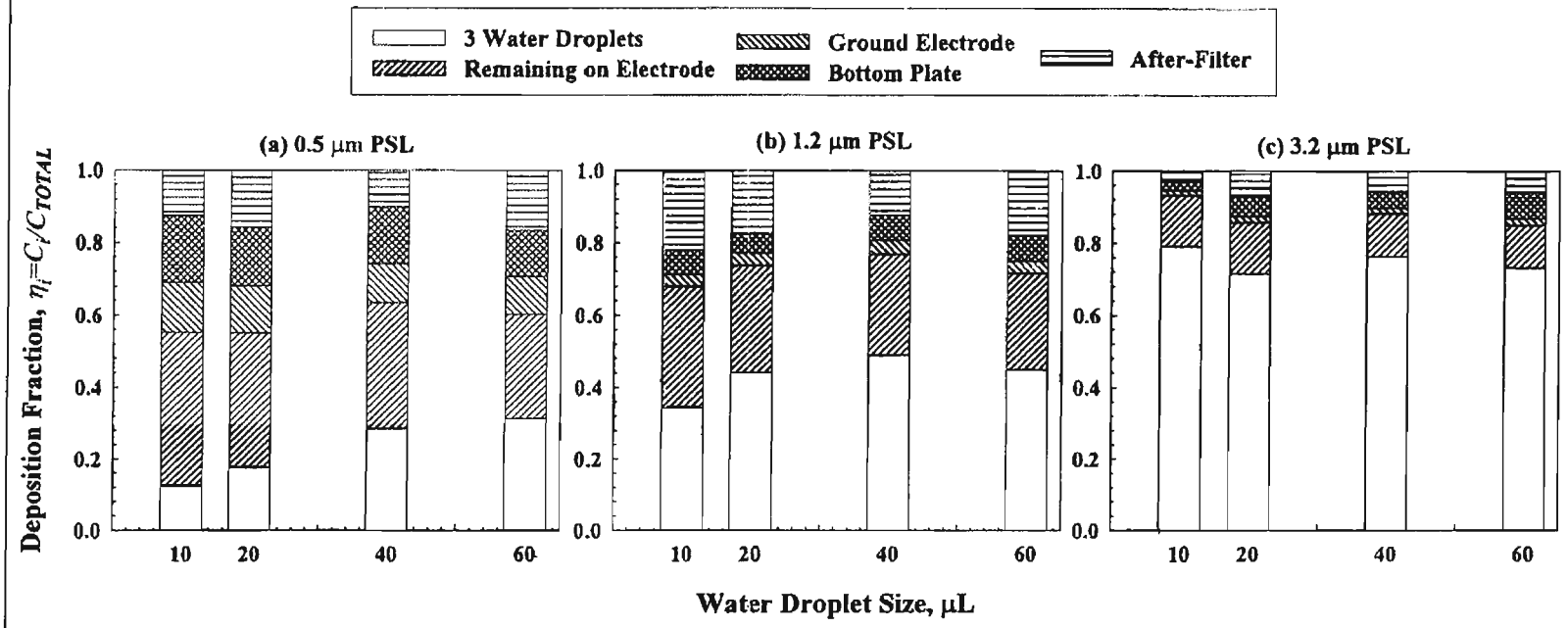

Figure 4.2. Deposition fraction for each component (3 water droplets, collection electrode, ground electrode, bottom surface, and after-filter) as a function of water droplet size (10, 20, 40, and 60 μL) when collecting PSL of (a) 0.5 μm, (b) 1.2 μm, and (c) 3.2 μm at 10 L/min flow rate and at the 12 V/50 mA charging condition and 7 kV collection voltage. The maximum standard deviation is 0.04 from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

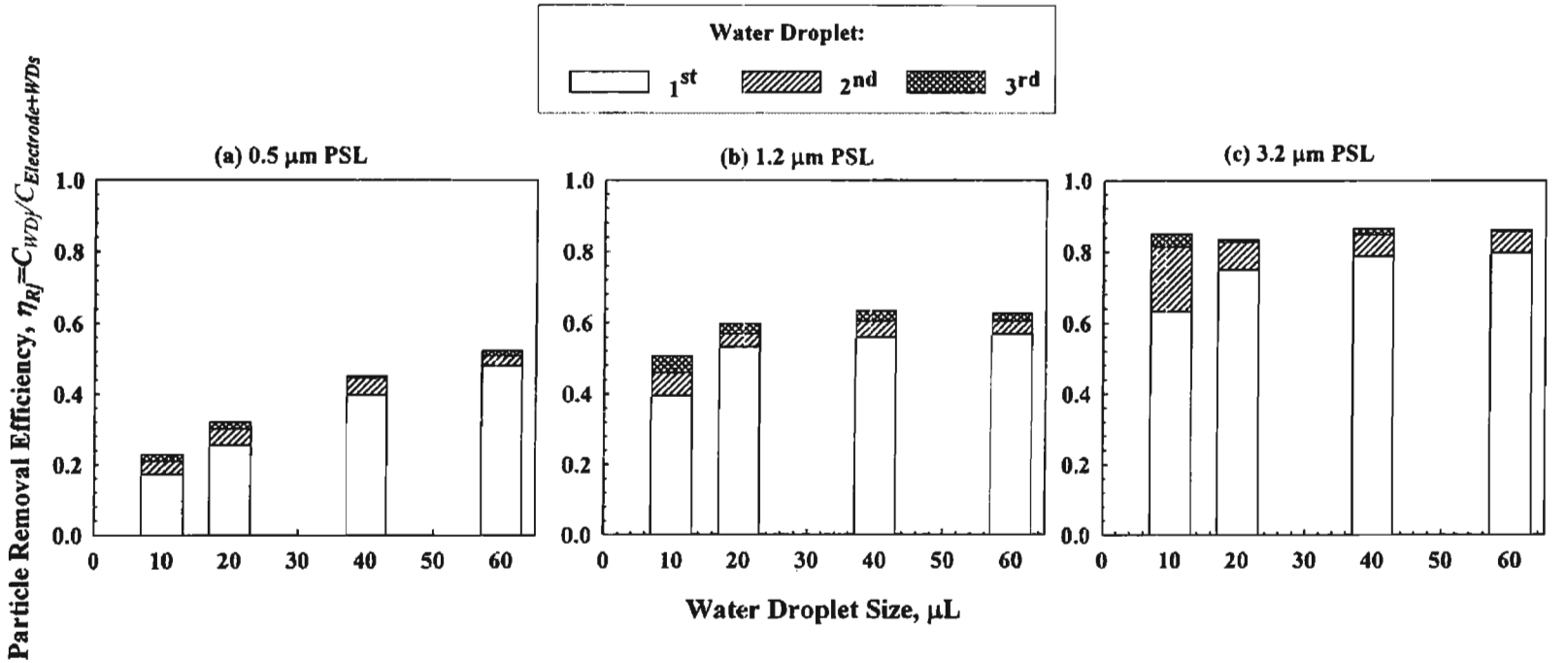

Figure 4.3. Efficiency of particle removal from the collection electrode by each sequential water droplet as a function of water droplet size (10, 20, 40, and 60 μL) when collecting PSL of 3.2 μm at 10 L/min flow rate and at the 12 V/50 mA charging condition and 7 kV collection voltage. The maximum standard deviation is 0.043 from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

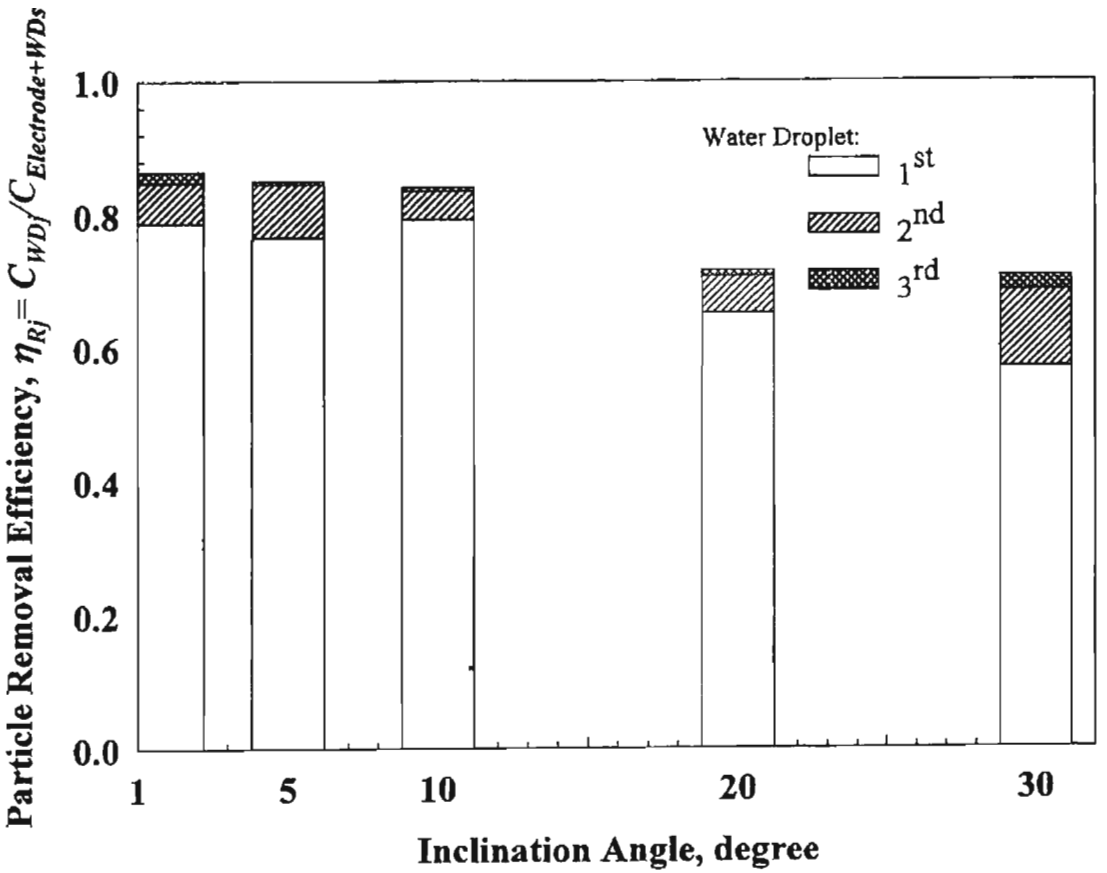

Figure 4.4. Efficiency of particle removal from the collection electrode by each sequential 40 µL water droplet as function of the sampler's inclination angle (1, 5, 10, 20, and 30 degrees) at 10 L/min flow rate and at the 12 V/50 mA charging condition and 7 kV collection voltage. The maximum standard deviation is 0.014 from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

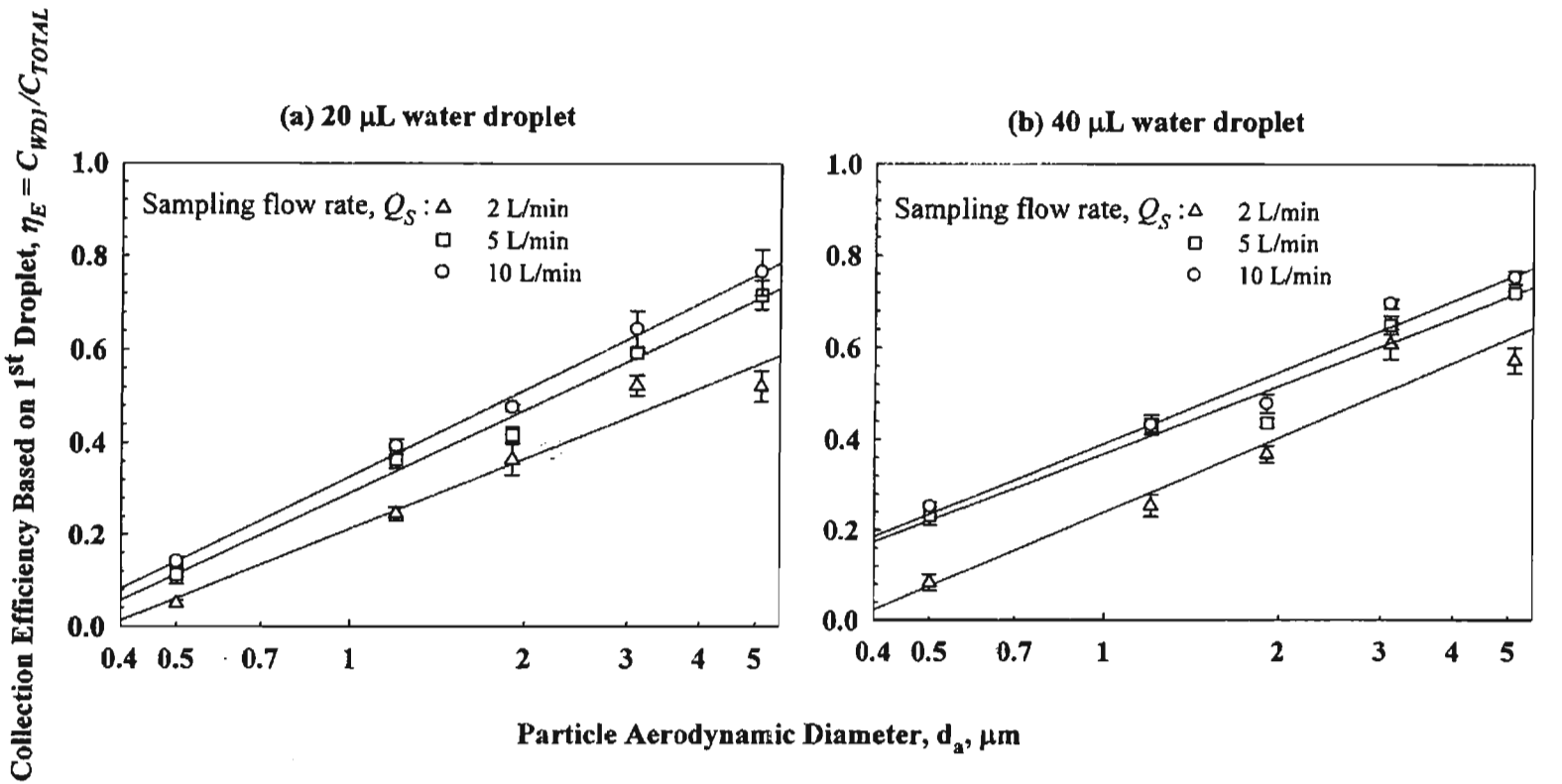

Figure 4.5. Sampler's collection efficiency based on the 1st water droplet (particles accumulated in the first droplet vs particles entering the sampler) as a function of particle size (0.5, 1.2, 1.9, 3.2, and 5.1 μm) at 2, 5, and 10 L/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage for two water droplet sizes: (a) 20 μL and (b) 40 μL. The error bars represent the standard deviations from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

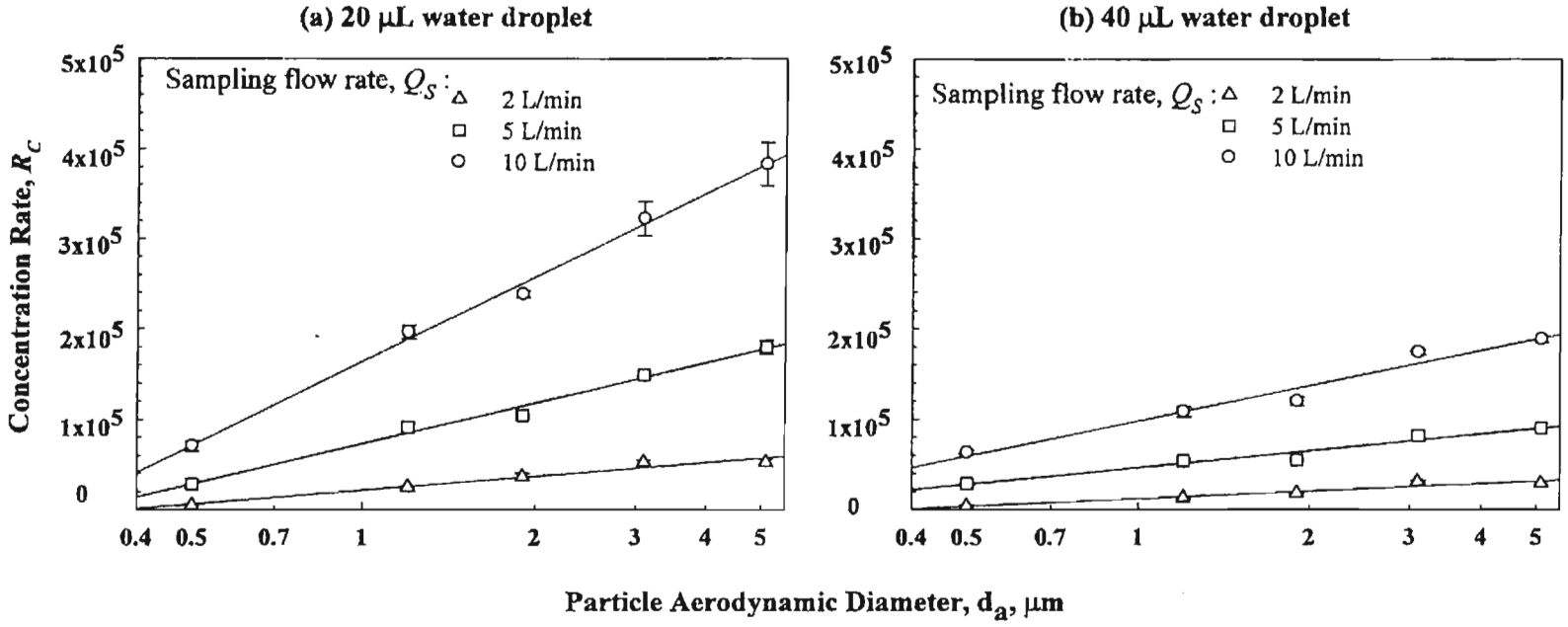

Figure 4.6. Sample concentration rates based on the 1st water droplet, as a function of particle size (0.5, 1.2, 1.9, 3.2, and 5.1 μm) at 2, 5, and 10 L/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage for two droplet sizes: (a) 20 μL and (b) 40 μL. The error bars represent the standard deviations from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

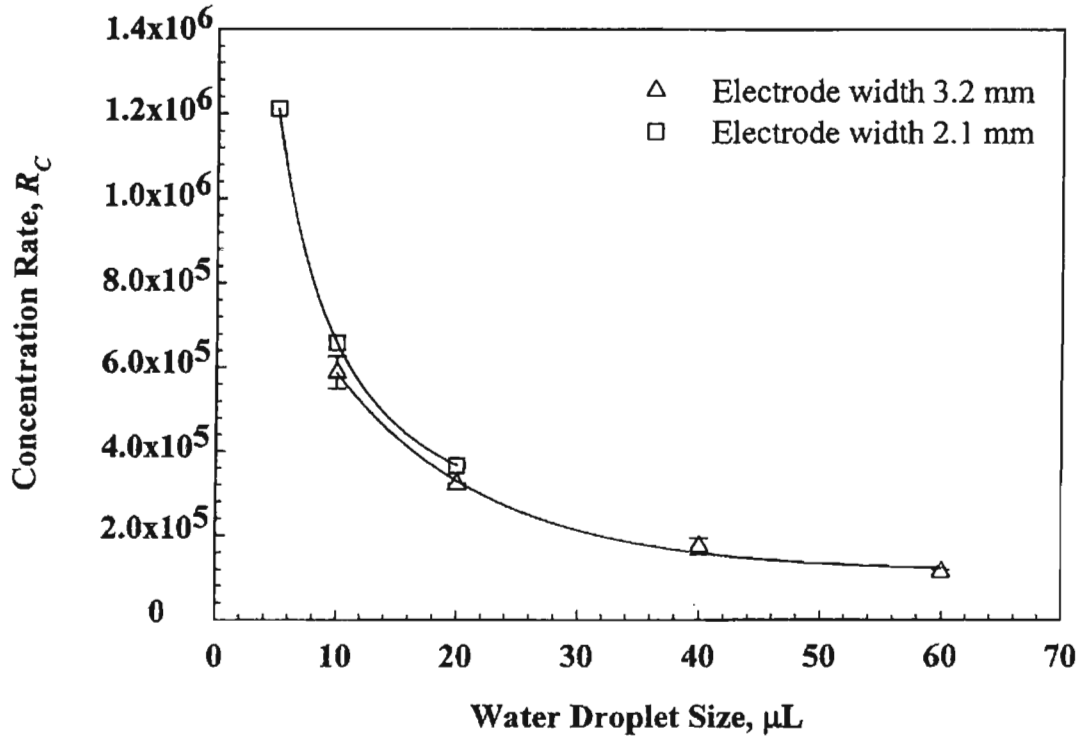

Figure 4.7. Comparison of sample concentration rates based on the 1st water droplet at two different collection electrode widths (3.2 versus 2.1 mm) as a function of droplet size (5, 10, 20, 40, and 60 µL) at 10 L/min flow rate and at the 12 V/50 mA charging condition and 7 kV collection voltage with 3.2 µm PSL. The error bars represent the standard deviations from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

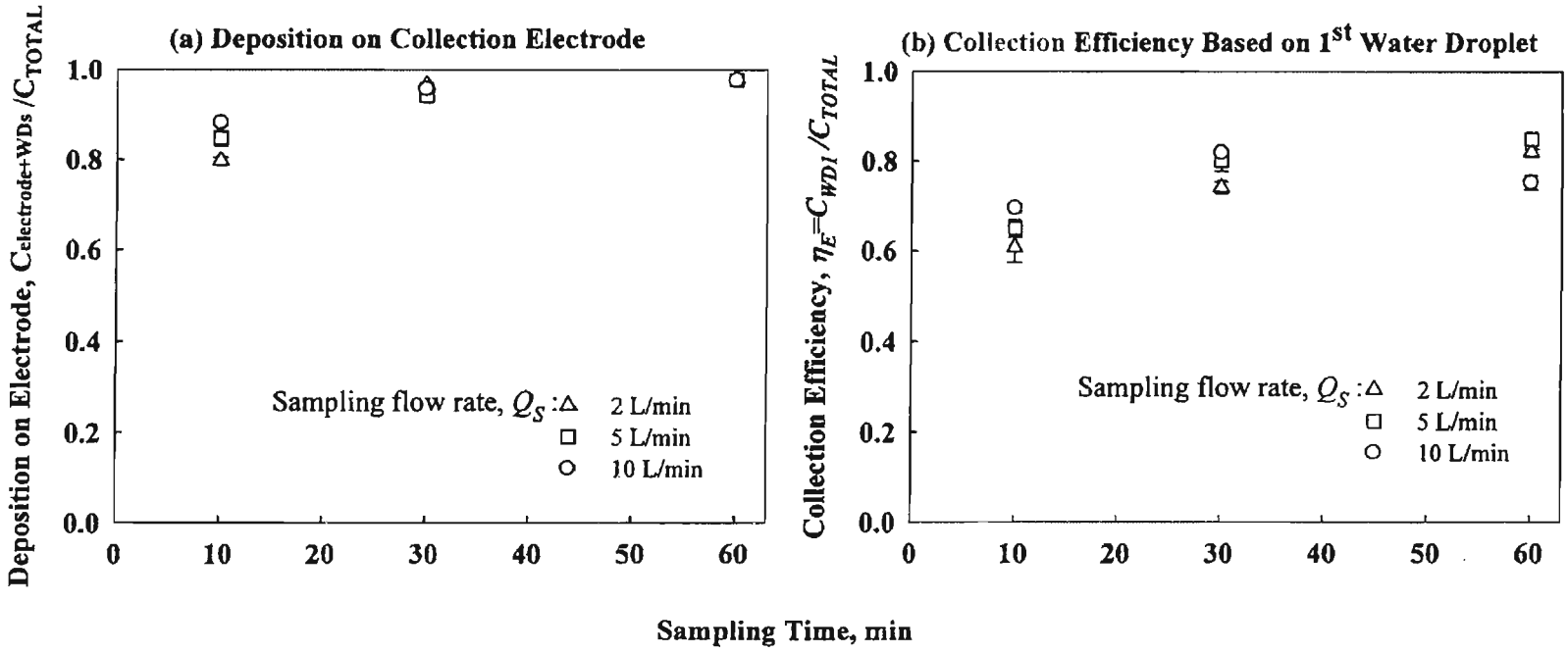

Figure 4.8. Sampler's performance as a function of sampling time (10, 30, 60 minutes) at 2, 5, and 10 L/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage with 3.2 μm PSL: (a) Fraction of particles deposited on collection electrode and (b) Collection efficiency based on 1st 40 μL water droplet. The error bars represent the standard deviations from 3 repeats.

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

Table 4.1. Overall relative precision of *COV*s for each particle deposition fraction in the electrostatic precipitator for experiments performed with 40 μL water droplet at 2, 5, and 10 L/min sampling flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage with 3.2 μm PSL.

Sampling Flow Rates	Deposition Fraction	*COV*, %		Mean *COV*, %	Standard Deviation of COV Values, %	Ratio of Standard Deviation to the Mean *COV*, *RP*
		Set1	Set2			
2 L/min	After-Filter	37.3	12.5	24.9	17.5	0.70
	Ground Electrode	8.8	14.1	11.5	3.8	0.33
	Bottom Surface	17.4	6.4	11.9	7.8	0.65
	Collection Electrode	19.7	14.7	17.2	3.5	0.21
	1st WD	5.4	3.7	4.5	1.2	0.26
	2nd WD	26.3	39.6	33.0	9.4	0.29
	3rd WD	10.3	13.5	11.9	2.3	0.19
5 L/min	After-Filter	17.6	26.9	22.3	6.6	0.30
	Ground Electrode	9.0	42.2	25.6	23.5	0.92
	Bottom Surface	10.0	4.3	7.1	4.0	0.56
	Collection Electrode	16.8	11.5	14.2	3.8	0.26
	1st WD	3.0	3.4	3.2	0.3	0.08
	2nd WD	34.6	12.8	23.7	15.4	0.65
	3rd WD	35.1	23.9	29.5	7.9	0.27
10 L/min	After-Filter	8.4	19.1	13.7	7.5	0.55
	Ground Electrode	7.7	5.9	6.8	1.3	0.19
	Bottom Surface	5.5	15.3	10.4	6.9	0.66
	Collection Electrode	15.9	15.3	15.6	0.4	0.03
	1st WD	1.5	2.4	1.9	0.6	0.33
	2nd WD	17.1	19.8	18.4	1.9	0.10
	3rd WD	44.9	52.7	48.8	5.5	0.11
				Average of all values		0.36

REF: Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

5 Analysis of sampler's collection efficiency when challenged with bacteria

The research described in this chapter responds to Specific Aim IV: "Analysis of physical collection efficiency using biological test particles". The main goal of this part of the study was to analyze the performance of the Electrostatic Precipitator with Superhydrophobic Surface (EPSS) when collecting bacterial aerosols. As a part of the experiments, we developed and applied the whole-cell QPCR technique to quantify the collected bacteria (additional Specific Aim). The data obtained using the whole-cell QPCR were compared with those obtained with a more traditional total cell counting method using acridine orange epifluorescence microscopy (AOEM). The settings of the EPSS collection voltage and charging conditions were the same as when testing with PSL particles and here its performance was tested as a function of the collecting water droplet size (5 and 40 μL), different sampling flow rates (2, 5, and 10 L/min) and the sampling time period (10, 30 and 60 min). The experiments were performed with vegetative cells of two different, commonly used test microorganisms: Gram-negative *Pseudomonas fluorescens* as a sensitive organism and Gram-positive *Bacillus subtilis* var. *niger*, as a hardy organism.

5.1 Biological Test Particles

The test microorganisms used in this study, *P. fluorescens* and *B. subtilis* are representatives of sensitive and hardy organisms, respectively, and are commonly found in indoor and outdoor environments (Hill et al., 1999; Johnson, 1994; Neidhardt et al., 1990). *P. fluorescens* (ATCC 13525) were obtained from the American Type Culture Collection (Rockville, MD) and grown in nutrient broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) at 26°C for 18 hours in a shaking incubator. After the growth, the cells were harvested by centrifugation at 7000 rpm (6140 g) for 5 min, at 4 °C (BR4, Jouan, Winchester, VA) and then washed 3 times with sterile deionized water under the same conditions. Prior to the experiments, the resulting cell pellet was resuspended in sterile deionized water to obtain suspension with the target cell concentration of ~10^9 cells per mL, as determined by the epifluorescence microscopy.

Dry *B. subtilis* spores were obtained from the US Army Edgewood Laboratories (Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD). The *B. subtilis* spores were suspended in sterile deionized water, and then activated at 60 °C for 25 min. The activated *B. subtilis* was cultured in nutrient broth for 18 hrs at 30 °C to obtain vegetative *B. subtilis*. Prior to the experiments, freshly grown vegetative *B. subtilis* were prepared using the same method as described above.

5.2 Experimental setup and sample collection

The experimental setup for this part of the study is shown in Figure 5.1.It is essentially the same as used in for testing with PSL particles. However, an alternative set-up was added to determine the bioaerosol reference concentration. The test bacteria were aerosolized using a 6-jet Collison nebulizer (BGI Inc., Waltham, MA) operated at a flow rate Q_A (4 L/min) and diluted with HEPA-filtered air flow Q_D (36 L/min). The 40 L/min aerosol stream was passed through a 2-mCi Po-210 charge neutralizer. The electrically neutralized bioaerosols then passed through a 0.035

m duct housing a vertically oriented ionizer (Wein Products Inc., Los Angeles, CA) which imparted a positive charge on the particles under controlled operating conditions (12V/50mA). The positively charged bioaerosols passed through a flow straightener and entered the test chamber where they were collected by the EPSS operated at a sampling flow rate, Q_S (2, 5, or 10 L/min). After a certain sampling time, t (10 to 60 min), the bacterial particles deposited on the superhydrophobic surface were removed by a rolling water droplet (5 or 40 μL) which was collected in a vial. The collected cell number was determined using the epifluorescence microscopy and the whole-cell QPCR assay as described below.

For each test, about 30 mL of fresh cell suspension was prepared for nebulization as described above. The 3.2 mm wide collection electrode was used for 40 μL droplets, while the 2.1 mm electrode was used for 5 μL droplets. Each collecting electrode of the EPSS was prepared by coating it with a superhydrophobic spray (HIREC-1450, NTT Corporation Inc., Japan) followed by drying at 60 °C for at least 1 hour. Before each coating, the electrode was thoroughly rinsed with ethyl acetate and then autoclaved. One stable DC power supply (BK Precision, Yorba Linda, CA) provided power to the ionizer, while another stable DC high voltage power supply (Bertan Associates, Inc, Valhalla, NY) provided collecting voltage to the EPSS. Voltages for the charger and collector were the same as used in the experiments with PSL particles.

5.3 Determination of total bacterial number by microscopy

The total number of cells collected by the EPSS and removed by the droplet was determined by acridine orange epifluorescence microscopy (AOEM) using the Axioskop 20 (Carl Zeiss MicroImaging Inc., Thornwood, NY). When counting cells using the epifluorescence microscopy, the droplet (5 or 40 μL) containing particles collected by the EPSS was diluted by adding sterile and purified water to increase the liquid volume to 1 mL. The resulting 1 mL sample was serially diluted in 10-fold dilutions with sterilized water to achieve a concentration comfortably countable using the epifluorescence microscopy. Each slide was prepared by filtering 1 mL aliquot of a selected dilution through a 25 mm black polycarbonate filter (Fisher Scientific, Suwannee, GA) and then staining it with 1 mL of 0.1 μg/mL Acridine Orange solution (Becton Dickinson Microbiology Systems, Sparks, MD) for 10 min. After washing with 3 mL of sterilized water and air-drying, at least 20 microscope fields were counted twice using the 100x oil-immersion objective. The total cell number in each sample, N_{sample}, was calculated as follows:

$$N_{sample} = N \times X \times D_{10-fold} \tag{5.1}$$

Here, N is the average cell count in each microscope view field, X is the number (X=6125) of fields for the entire filter, and $D_{10\text{-}fold}$ is the dilution factor.

5.4 Development of whole-cell quantitative real-time PCR method to quantify the collected bacteria

In addition to analyzing the samples with acridine orange epifluorescence microscopy (AOEM), we developed and applied the whole-cell quantitative real-time PCR (whole-cell QPCR) assay.

When a biological sample is analyzed by the QPCR, the output from the reaction is a threshold cycle (C_T) value which is defined as the PCR cycle number at which the fluorescence of the

amplicon exceeds a calculated threshold value. The C_T value is inversely proportional to the initial DNA (or cell) concentration with which the reaction was started. Thus, a standard (calibration) curve could be prepared relating known cell concentrations (determined by the epifluorescence microscopy) with the C_T values.

Our earlier research indicated that the standard curves should be study-specific, i.e., they should be prepared using the same sampling protocol that will be used to collect actual samples (An et al., 2006). In addition, to increase the labor and time-efficiency of the method, we hypothesized that one could use whole-cell QPCR method with a universal primer, i.e., unlike in the conventional PCR where DNA has to be extracted from cells prior to the PCR reaction, in this method, aliquots from untreated cell suspensions would be directly used in the PCR reaction as templates. Absence of the DNA extraction step increases efficiency of the method.

When preparing the standard curves for the whole-cell QPCR, the volume of samples collected by the EPSS (5 μL) was increased to 1 mL. From this 1 mL sample suspension, 100 μL aliquot was transferred to a sterilized microcentrifuge tube and serially diluted in 10-fold water-based dilutions ranging from 10^0 to 10^{-5} until concentration countable by the microscopy was achieved. The number of cells in each dilution was determined by the epifluorescence microscopy as described above and then related to the whole-cell QPCR output (C_T value) at each dilution. During the PCR reaction, 5 μL aliquots of each dilution were directly (without extracting genomic DNA first) used as templates and the reactions were performed in triplicate. QPCR amplification was performed using iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) using the iQ SYBR Green supermix PCR Kit (Bio-Rad Laboratories, Hercules, CA). Amplification and detection of DNA by real-time PCR was performed on iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) using the iQ SYBR Green supermix PCR Kit (Bio-Rad Laboratories, Hercules, CA) consisting of 2× SYBR Green supermix (with the hot-start enzyme, iTaq™ DNA polymerase, SYBR PCR buffer, dNTP mix, SYBR I, 20 nM fluorescein for dynamic well factor collection, and 6 mM $MgCl_2$). Reaction mixes were prepared by combining 12.5 μL of 2× SYBR Green supermix, primers and H_2O to a total volume of 25 μL for each reaction. The universal primers originally described by (Nadkarni et al., 2002) including the forward primer, 5'-TCCTACGGGAGGCAGCAGT-3' and the reverse primer, 5'-GGACTACCAGGGTATCTAATCCTGTT-3' were used at final concentration of 0.25 μM for each primer and produced 466bp amplicon (between residues 331 and 797 on the *E. coli* 16S rRNA gene). Amplification reaction was performed using the following program with iCycler iQ™ thermal cycler (Bio-Rad Laboratories, Hercules, CA): 10 min at 95 °C; 40 cycles of (15s at 95 °C 1 min at 60 °C). Data analysis was performed using iCycler iQ™ Real–Time detection system software. After completing the PCR amplification cycles, a melt curve was generated for the resulting amplicon by measuring loss of fluorescence over a temperature range of 55-92 °C. If there is a contamination during the reaction, a peak, other than desired amplicon peak, would appear in melting curve thus indicating the contamination of DNA, non-specific binding, or occurrence of primer dimers. Additional information about the primer set and PCR amplification conditions are provided in our previous study (An et al., 2006). Non-specific PCR products from bacterial samples, such as primer dimers or false priming amplicons were not observed in melt curves. If a negative control (no template added in PCR reaction) had C_T values of ≤30, the C_T values from the entire reaction were discarded. The standard curves and the resulting equations

obtained for the *P. fluorescens* and *B. subtilis* bacteria are shown in Figure 5.2. Each point represents an average and standard deviation of triplicate C_T values.

When analyzing performance of the EPSS using the QPCR method, the collected bacteria were removed by a 5 μL droplet and the droplet was used directly in the PCR reaction as a template. The number of collected cells, N_{sample}, was determined using the corresponding C_T values and equations shown in Figure 5.2:

$$N_{sample} = f(C_T) \tag{5.2}$$

5.5 Determination of the EPSS collection efficiency

Before and after each measurement with the EPSS, the reference concentration of the airborne bacteria was determined by an Aerodynamic Particle Sizer (APS, Model 3321, TSI Inc., Shoreview, MN) and an isokinetic probe as shown in bottom left of Figure 5.2. The accuracy of the reference concentration measurements was verified by comparing the bacterial concentrations determined by the APS with those measured using a reference filter. Here, the bacteria were isokinetically collected on a 47 mm membrane filter (Pall Inc., East Hills, NY) for 10 min and were then extracted into liquid using the procedure reported by Wang et al. (2001): the filter was soaked in 30 mL of sterile deionized water for 10 minutes, followed by vortexing for 2 minutes and sonicating for 15 minutes. The number of bacteria in the resulting suspension was determined using epifluorescence microscopy. It was found that the two number concentrations (APS reading versus reference filter) agreed within 8%. Given the inherent uncertainty in microorganism counting by microscopy (standard deviation is usually ~20%), this was an acceptable agreement. Thus, the collection efficiency, η, of the EPSS for each sampling condition was defined as:

$$\eta = \frac{N_{sample}}{N_{reference}} = \frac{N_{sample}}{R_{APS} \times 1000 \times Q \times t} \tag{5.3}$$

Here, N_{sample} is the number of cells in a water droplet determined either by microscopy or whole-cell QPCR, $N_{reference}$ is the reference cell number measured by the APS, R_{APS} is the average cell concentration (#/cm^3) which is measured by the APS every 6 seconds for 150 seconds before and after each sampling, Q_s (L/min) is the sampling flow rate of the EPSS, and t (min) is the sampling time period.

In addition to the collection efficiency, we also determined the concentration rate, R_C, which represents the ratio of particle concentration in liquid versus the concentration of particles in air per time period. The concentration rate R_C with units of min^{-1} could be expressed as follows:

$$R_C = \frac{Q_S}{V_{WD}} \times \eta \tag{5.4}$$

where Q_s (L/min) is the sampling flow rate and V_{WD} (L) is the volume of the collecting water droplet. Since our earlier part of the investigation showed that the majority of particles deposited on the collection electrode are removed by the 1st water droplet, the collection efficiency and the concentration rate were calculated based on the 1st water droplet.

5.6 Results and discussion

The representative number concentrations and particle size distributions of airborne *P. fluorescens* and *B. subtilis* bacterial cells measured by the APS are shown in Figure 5.3. As could be seen, the mean aerodynamic diameters, d_{ae}, of the bacteria were 0.82±0.02 μm and 0.89±0.03 μm, respectively, and the particle agglomerates were absent.

Figures 5.4 and 5.5 present the collection efficiency and the resulting concentration rate of the EPSS, respectively, when sampling the two bacteria at different sampling flow rates (2, 5, and 10 L/min) and using water droplets of two different volumes (5 and 40 μL) to remove the collected particles. The sampling time in these tests was 10 minutes and the number of collected particles was determined using epifluorescence microscopy. As could be seen from Figure 5.4, the collection efficiency of the EPSS when using 40 μL collection droplets ranged from 52% to 61% for *P. fluorescens* (d_{ae} = 0.82 μm) across the sampling flow rates. For *B. subtilis* (d_{ae} = 0.89 μm), the collection efficiencies ranged from 59% to 72% for the same sampling flow rates. When the droplet of 5 μL was used, the collection efficiency averaged over all three sampling flow rates and both bacteria decreased by approximately 7% compared to the 40 μL droplet. For both sampling droplet sizes the collection efficiency was somewhat higher at the higher flow rates (10 L/min) compared to the lower flow rates (2 L/min). A similar pattern was also observed when testing with the PSL particles we it attributed to lower particle losses inside the EPSS when sampling at higher flow rates. However, the actual EPSS collection efficiency value when sampling the PSL particles of similar sizes and the same sampling flow rates was lower and ranged from 10 to 40%. Clearly, the EPSS collects the bacterial particles more efficiently than the PSL particles of the same size. The observed efficiency with which the particles entering the EPSS are removed from the air stream is approximately the same for bacteria and PSL. Thus, higher collection efficiency for bacteria is likely achieved by their more efficient removal from the electrode due to weaker adhesion forces between the bacteria and the electrode compared to the adhesion forces for the PSL particles. The tests presented in Figure 5.4 were conducted in triplicate and the maximum standard deviation was 0.08.

Figure 5.5 presents the concentration rates based on the collection efficiency data presented in Figure 5.4. As could be seen, for the 40 μL collection droplet and the sampling flow rate of 10 L/min the concentration rate for *P. fluorescens* was 150,000 and reached 180,000 for *B. subtilis*. These values are higher compared to the concentration rates of PSL particles of similar size, due to higher collection efficiency for bacteria. For 5 μL droplet and the sampling flow rate of 10 L/min we achieved sample concentration rates as high as 1.2 million for both bacteria.
For many bioaerosol sampling applications sampling times longer than 10 minutes are needed. Therefore, in addition to the 10 min sampling time data, performance of the EPSS was tested for sampling times of 30 and 60 min and the data for *P. fluorescens* and *B. subtilis* are presented in Figures 5.6 and 5.7, respectively.

When sampling of *P. fluorescens* for 30 min was performed with 5 μL droplet and the flow rates were increased from the lowest (2 L/min) to the highest (10 L/min), the collection efficiency increased from 34±3% to 47±3%; for 60 min sampling, the collection efficiency increased from 18±3% to 24±3% (Figure 5.6). However, compared to the 10 min sampling, the collection efficiency after 30 and 60 min decreased by 22% and 60%, respectively, when averaged for all sampling flow rates. The trend for 40 μL water droplet was similar: the collection efficiency

decreased by 33% and 53% for 30 and 60 min sampling time, respectively, compared to 10 min sampling.

The data for *B. subtilis* presented in Figure 5.7 show a trend similar to that of *P. fluorescens*. When sampling for 30 and 60 min, the collection efficiencies with both 5 and 40 μL water droplets have slightly increased at 10 L/min compared to 2 L/min. However, compared to the 10 min sampling, the collection efficiency decreased for 30 and 60 min sampling, although the decrease was less steep compared to the *P. fluorescens* bacteria. For 5 μL water droplet, after 30 and 60 min of sampling, the collection efficiency decreased on average by 17% and 26%, respectively, compared to 10 min sampling time. The average decrease for 40 μL water droplet was by 21% and 31% for 30 and 60 min sampling time, respectively.

The observed decrease in the collection efficiency for both bacteria with increasing collection time is opposite compared to the experiments with PSL particles, where at longer sampling times the collection efficiency either stayed the same or slightly increased. One possible explanation could be "saturation" of the water droplet by a higher number bacteria deposited on the electrode after a longer sampling time. However, the concentrations of both bacteria were adjusted so that the total number of bacteria deposited inside the EPSS would be approximately the same for all three sampling times. Thus, the most likely explanation of the observed result is a less efficient removal of the deposited bacteria by the water droplet after their prolonged contact with the collection electrode, e.g. increase in the adhesive force with time. The adhesive force between the particles and the surface depends on the particle type, net electrical charge and the contact time (Hinds, 1999). Although the superhydrophobic surface is supposed to provide a very small surface area contact with the particles (this phenomenon allows for a high water contact angle), it could be expected that occurring physical phenomena will be similar to other surfaces, i.e., electrostatic and van der Waals forces would gradually deform the asperities and increase the contact area thus increasing the adhesion forces (Hinds, 1999).

The collection efficiency of the EPSS was also determined by quantifying the collected cells using the whole-cell QPCR to investigate compatibility of the EPSS with this sample analysis method. Here, an additional set of tests was carried out at a 10 L/min sampling flow rate, 10 minute sampling time and the bacteria deposited on the sampler's electrode were collected using 5 μL water droplets. The voltages for particle charging and collection remained the same as in the previous tests.

For each bacterium, the reference cell number as measured by the APS, the number of collected cells determined using the QPCR and the epifluorescence microscopy, as well as the resulting collection efficiency are presented in Table 5.1. The presented data also show uncertainty of the measurement for each replicate. When calculating the collection efficiency, the error was propagated. The collection efficiency and the resulting concentration rate are also graphically shown in Figure 5.8. As could be seen in Table 5.1 and Figure 5.8, the average collection efficiency of *P. fluorescens* determined by the QPCR was 66±4%: approximately 15% higher compared to the average collection efficiency determined by microscopy. For *B. subtilis*, a hardy gram positive bacterium, the collection efficiency determined by microscopy (65±7%) was higher than the average collection efficiency determined by the whole-cell QPCR (54±3%). However, based on the *t-test*, the difference in the average collection efficiencies when taking

into account propagated error was not statistically significant for either *P. fluorescens* or *B. subtilis*: p=0.38>0.05 and p=0.20>0.05, respectively. The data presented in Figure 5.8 show that the concentration rate averaged for both microorganisms and both detection methods was ~$1.2x10^6$/min. Thus, the EPSS can efficiently operate at a sampling flow rate of 10 L/min with about 30-500 times higher concentration rate as compared to other liquid samplers (e.g., Biosampler, BioGuardian, BioCapture, etc.

As could be observed from Figure 5.4a and Table 5.1, the average collection efficiency determined using cell counting by microscopy (AOEM/APS) for the two separate test sets was the same for *B. subtilis*, but slightly different for *P. fluorescens*: 58% versus 51%. However, this difference was not statistically different: P=0.27>0.05.

The data presented in Table 5.1 and Figure 5.8 show that the samples collected by the EPSS could be effectively analyzed using the whole-cell QPCR method which offers certain advantages over conventional QPCR. The whole-PCR method developed here does not require the DNA extraction prior to the PCR reaction and is simpler and easier to use compared to the traditional QPCR. It is also labor and time-efficient compared to microscopy and allows processing more samples over the same time period, while being suitable to measure particle concentrations that are too low to determine using microscopy (An et al., 2009).

The EPSS has achieved very high concentration rates when sampling two test bacteria. However, for other particles, its performance may vary depending on the nature of particles, their hydrophobicity, and the interaction between particles and the collection surface.

We also compared the reproducibility of the collection efficiency data obtained by the two different methodologies (AOEM versus QPCR) by calculating the coefficient of variation (*COV*) for each sample. As could be seen from Table 5.1, the mean *COV* value ($\overline{COV}$) of QPCR data was 11% for both *P. fluorescens* and *B. subtilis*, indicating tightly grouped, precise data. However, the AOEM data sets had a $\overline{COV}$ of 27% for *P. fluorescens* and 21% for *B. subtilis* indicating that the data were spread over a wider range of values. This is due to the inherently higher uncertainty in cell counting by microscopy. Overall, we believe that the whole-cell QPCR provides a promising alternative to the microscopic counting method in bioaerosol quantification. One potential drawback is the need for study- and microorganism-specific standard curves. However, once a pool of standard curves for different organisms and various sampling protocols is obtained, we believe the average value could be used to get a reasonably good estimate of the airborne microorganism concentration.

5.7 Figures and tables for Chapter 5

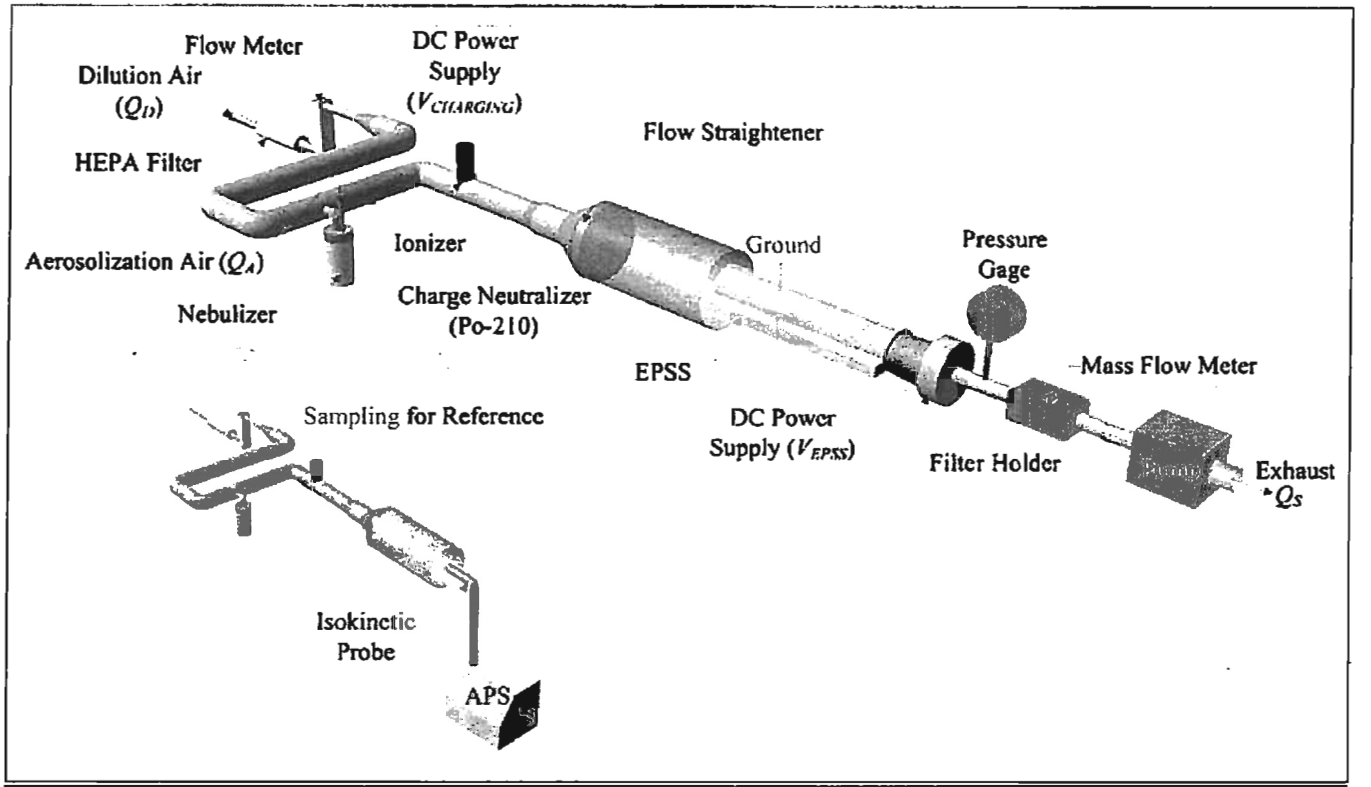

Figure 5.1. Schematic diagram of the experimental setup. The figure in left bottom corner shows an alternative set-up used to determine the bioaerosol reference concentration.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

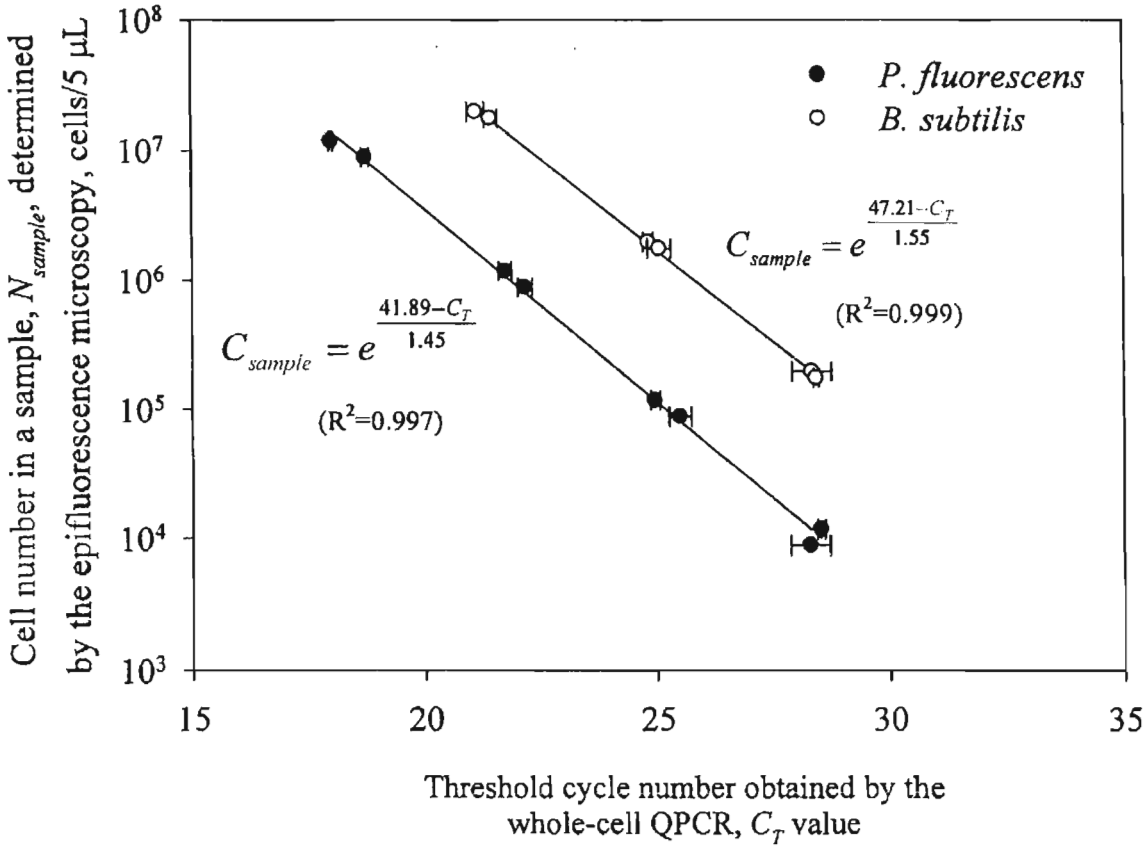

Figure 5.2. Standard curves of *P. fluorescens* and *B. subtilis* based on the relationship between the C_T values and the bacterial concentration as determined by the epifluorescence microscopy.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

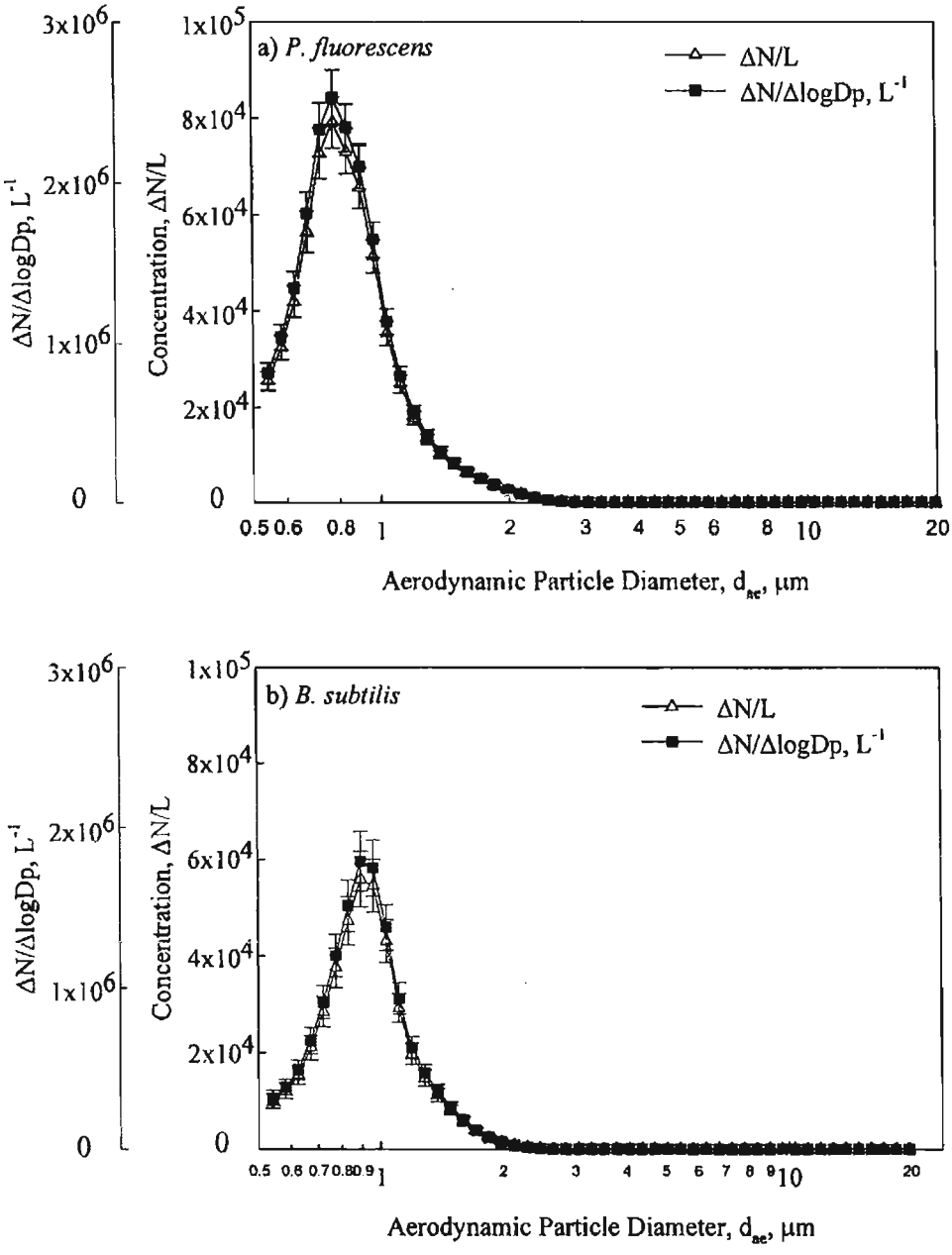

Figure 5.3. Particle size distributions according to the number concentration (ΔN/L) and the normalized concentration (ΔN/ΔlogDp, L^{-1}) for two biological particles: (a) *P. fluorescens* and (b) *B. subtilis*.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

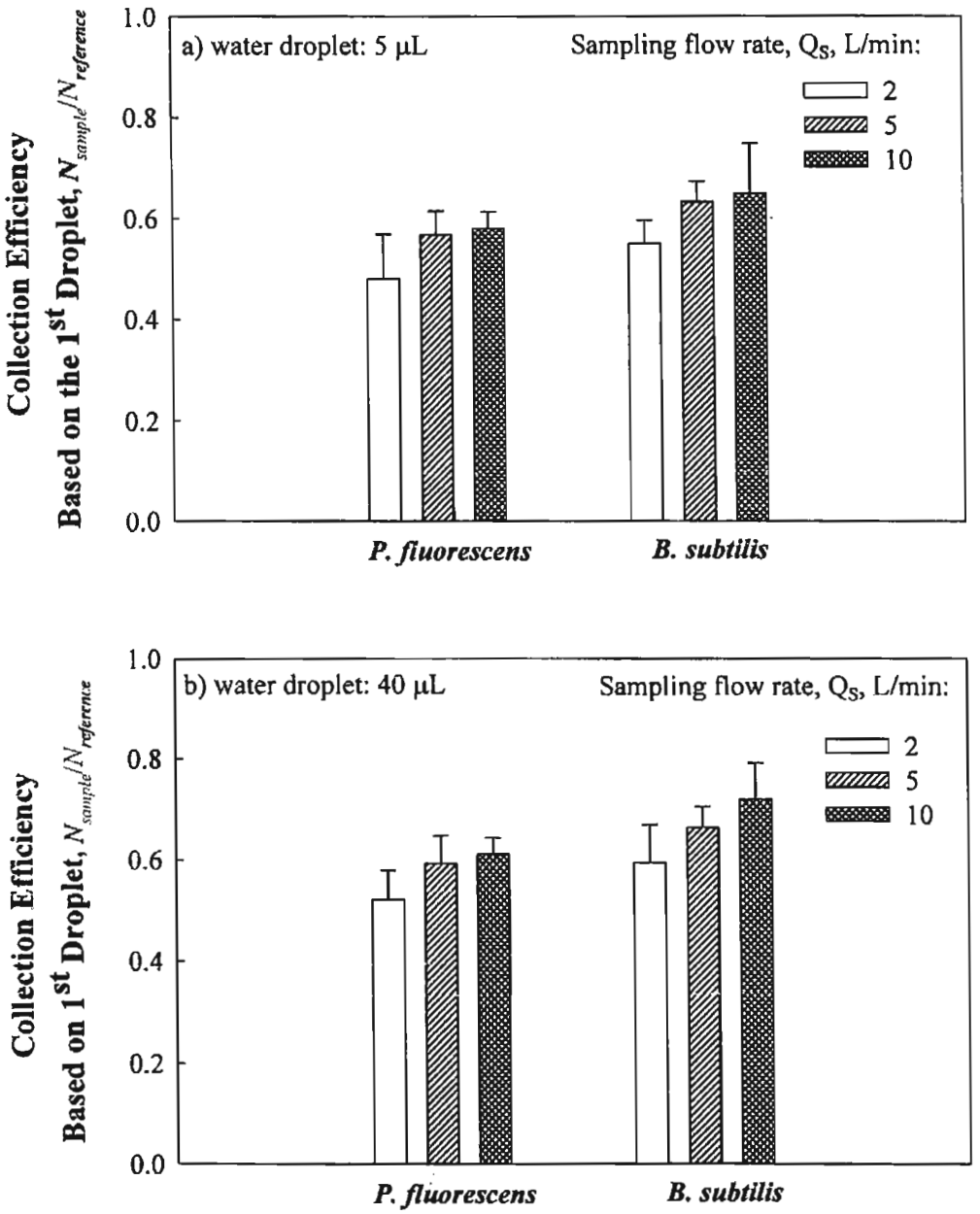

Figure 5.4. The collection efficiency of *P. fluorescens* and *B. subtilis* bacteria for different volumes of collecting water droplet, (a) 5 μL and (b) 40 μL, at three different sampling flow rates (2, 5, and 10 L/min) and at the 12V/50 mA charging condition and 7kV collection voltage. The maximum standard deviation is 0.10 from three repeats.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

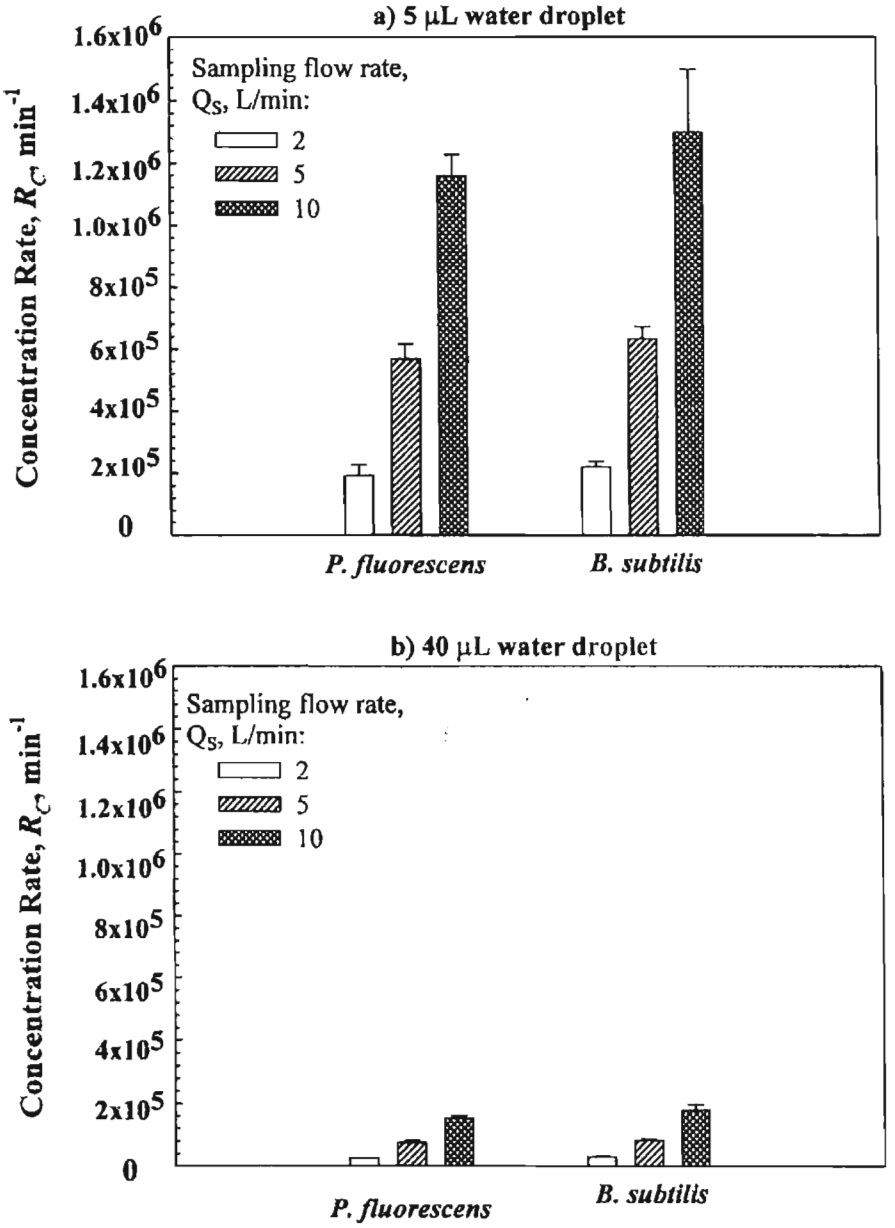

Figure 5.5. Comparison of the concentration rates based on the 1st water droplet as a function of droplet size, (a) 5 μL and (b) 40 μL, at 2, 5, and 10 L/min sampling flow rates and at the 12V/50 mA charging condition and 7kV collection voltage with *P. fluorescens* and *B. subtilis*. The error bars represent the standard deviation from three repeats.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

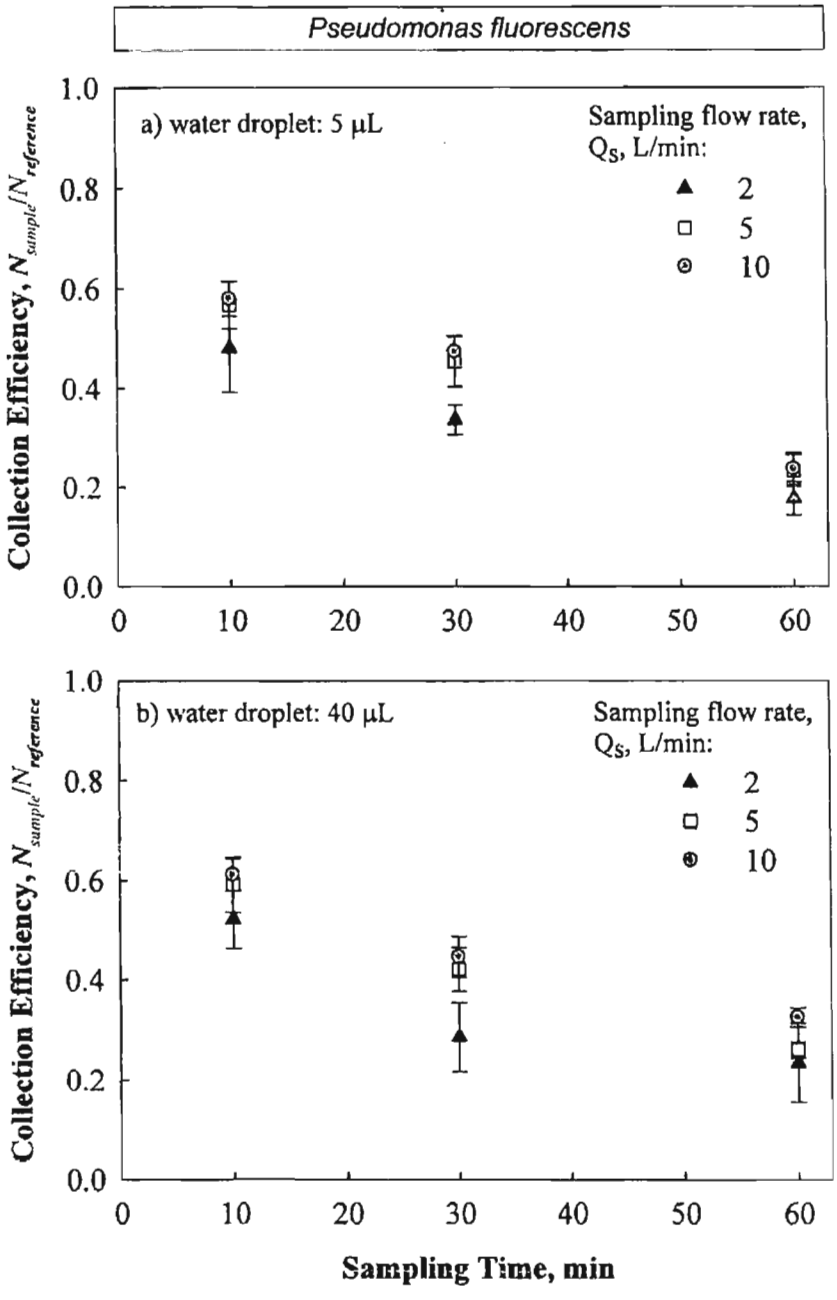

Figure 5.6. Collection efficiency based on the 1st water droplet, (a) 5 µL or (b) 40 µL, as a function of sampling time (10, 30, and 60 min) at 2, 5, and 10 L/min flow rates and at the 12V/50mA charging condition and 7kV collection voltage with *P. fluorescens*. The error bars represent standard deviations from three repeats.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

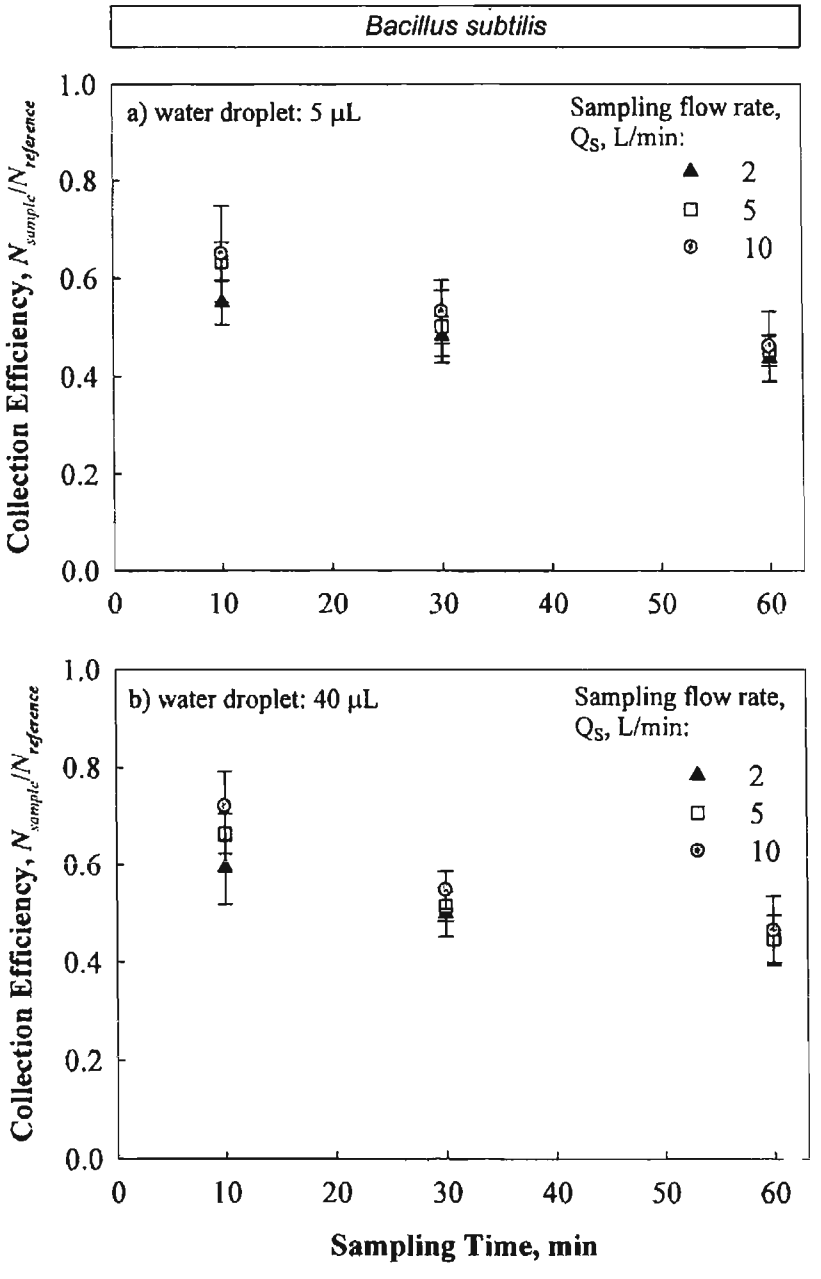

Figure 5.7. Collection efficiency based on the 1st water droplet, (a) 5 µL or (b) 40 µL, as a function of sampling time (10, 30, and 60 min) at 2, 5, and 10 L/min flow rates and at the 12V/50mA charging condition and 7kV collection voltage with *B. subtilis*. The error bars represent standard deviations from three repeats.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

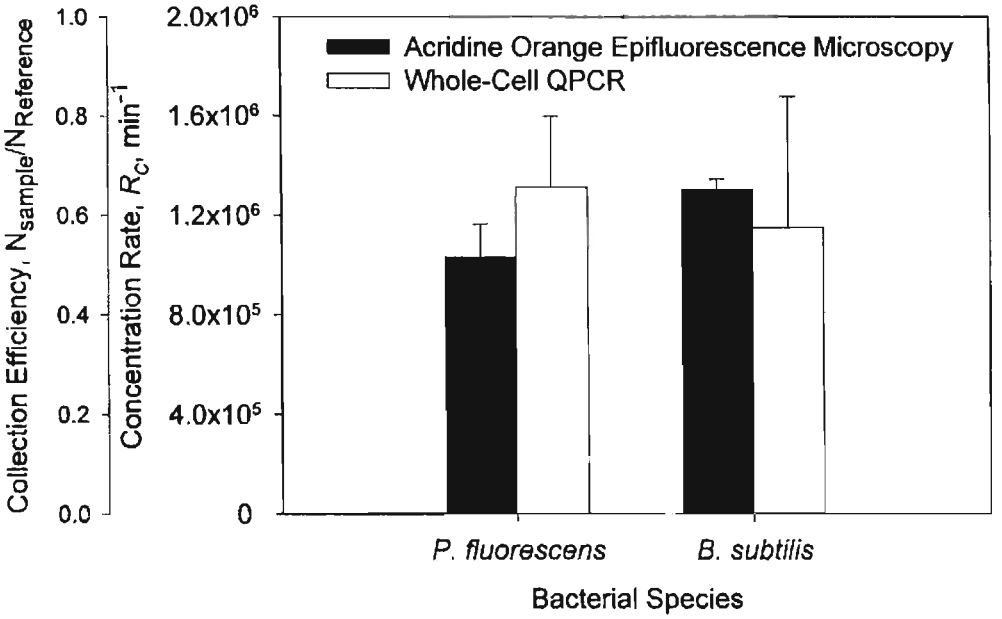

Figure 5.8. Comparison of collection efficiency of the EPSS based on the bacterial concentration determined by different quantification methods; black bars present data based on the acridine orange epifluorescence microscopy (AOEM) and white bars present data based on whole-cell QPCR method.

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

Table 5.1. Collection efficiency of the EPSS for *P. fluorescens* and *B. subtilis* bacteria based on two different methods to quantify the collected bacteria. The data are presented as average ± standard deviation.

Sample	Reference cell number by the APS	Total cell number determined by the AOEM	Total cell number determined by the whole-cell QPCR	Collection efficiency, AOEM/APS, %	Collection efficiency, whole-cell QPCR/APS, %
P. fluorescens					
Sample 1	$(3.78 \pm 0.17) \times 10^6$	$(1.72 \pm 0.47) \times 10^6$	$(3.06 \pm 0.42) \times 10^6$	46 ±13	81 ± 12
Sample 2	$(3.88 \pm 0.09) \times 10^6$	$(1.96 \pm 0.40) \times 10^6$	$(1.84 \pm 0.20) \times 10^6$	51 ±10	47 ± 5
Sample 3	$(3.78 \pm 0.18) \times 10^6$	$(1.83 \pm 0.52) \times 10^6$	$(2.47 \pm 0.31) \times 10^6$	48 ±14	65 ± 9
Sample 4	$(3.93 \pm 0.13) \times 10^6$	$(2.38 \pm 0.76) \times 10^6$	$(2.72 \pm 0.14) \times 10^6$	61 ±19	70 ± 4
	Average Collection Efficiency of the EPSS, %			51 ± 7	66 ± 4
	Average Coefficient of Variation (%), $\overline{COV}$			27	11
B. subtilis					
Sample 1	$(2.14 \pm 0.14) \times 10^6$	$(1.43 \pm 0.26) \times 10^6$	$(1.28 \pm 0.08) \times 10^6$	67 ±13	60 ± 5
Sample 2	$(2.37 \pm 0.08) \times 10^6$	$(1.50 \pm 0.27) \times 10^6$	$(1.22 \pm 0.06) \times 10^6$	63 ±12	51 ± 3
Sample 3	$(2.21 \pm 0.11) \times 10^6$	$(1.47 \pm 0.34) \times 10^6$	$(1.22 \pm 0.20) \times 10^6$	67 ±16	55 ± 9
Sample 4	$(2.61 \pm 0.068) \times 10^6$	$(1.66 \pm 0.34) \times 10^5$	$(1.24 \pm 0.15) \times 10^6$	64 ±13	48 ± 6
	Average Collection Efficiency of EPSS, %			65 ± 7	54 ± 3
	Average Coefficient of Variation (%), $\overline{COV}$			21	11

REF: Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

6 *Analysis of sampler's collection efficiency when challenged with fungi*

The research described in this chapter responds to Specific Aim IV: "Analysis of physical collection efficiency using biological test particles". The main goal of this research part was to investigate performance of the Electrostatic Precipitator with Superhydrophobic Surface (EPSS) when collecting fungal spores. As a part of the experiments, we developed and applied the ATP-based bioluminescence technique to quantify the collected spores (additional Specific Aim). The EPSS was challenged with three commonly found fungal spores - *Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor* and its performance was tested as a function of the collecting water droplet size (10 and 40 μL), airborne fungal concentration (10^0 - ~10^4 per liter) and the sampling time (10, 30 and 60 min). In addition, conditions resulting in the optimum performance of the ATP method for fungi analysis, such as the age of the culture prior to harvesting, were also investigated.

6.1 Fungal species used for testing

The fungal species used in this study, *Cladosporium cladosporioides* (ATCC 5899), *Penicillium melinii* (ATCC 10469), and *Aspergillus versicolor* (ATCC 26644), are common in indoor and outdoor environments (Horner et al., 1995; Madelin, 1994).. These fungal species were obtained from the American Type Culture Collection (Rockville, MD). All cultures were plated onto Sabouraud Dextrose Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated at room temperature (ca. 26°C) for seven days. During the optimization of the ATP method, the incubation time varied from 7 to 21 days. After incubation, about 3 mL of sterile deionized water was added to each plate and the spores from were gently harvested from mycelium using a spreader. The suspension volume was increased to 50 mL,and the spores were harvested by centrifugation at 5050g for 5 min, at 4 °C (BR4, Jouan, Winchester, VA) and then washed 5 times with sterile deionized water under the same conditions. This method of spore preparation was used in our earlier studies (Yao and Mainelis, 2006a). The resulting spore pellet was resuspended in sterile deionized water and then diluted 10-fold several times so that after aerosolization the target airborne concentration of ~10^2 -10^3 spores/L for most of the experiments, as determined by an Aerodynamic Particle Sizer (APS, Model 3321, TSI Inc., Shoreview, MN), could be achieved. The final spore suspension was examined under a microscope to verify that the vast majority (>95%) of particles are spores with very few fragments of mycelium

6.2 Experimental setup and sample collection

The experimental setup used in this part of research is shown in Figure 6.1. It is essentially the same setup as described in Chapter 5 and shown in Figure 5.1. The main difference was that an Aerodynamic Particle Sizer (APS, 3320, TSI, Inc., Shoreview, MN) was operated in parallel to the EPSS and isokinetically measured spore concentrations in the test chamber. The measurements by the APS were used as a reference when determining collection efficiency of the EPSS. For each test, about 30 mL suspension of the fresh fungal spores in sterile deionized water were aerosolized using a Collison nebulizer (BGI Inc., Waltham, MA), operated at a flow rate Q_A (4 L/min). After aerosolization, the spore-laden air was diluted with HEPA-filtered air

flow Q_D (36 L/min). The resulting aerosol stream of 40 L/min was passed through a 2-mCi Po-210 charge neutralizer. The electrically neutralized bioaerosols then passed through a 0.035 m duct housing a vertically oriented ionizer (Wein Products Inc., Los Angeles, CA), which imparted a positive charge on the particles under the same operating conditions as described above($V_{CHARGER}$ = 12V/50mA). A stable DC power supply (BK Precision, Yorba Linda, CA) provided power to the ionizer. The positively charged bioaerosol particles passed through the flow straightener and then entered the cylindrical test chamber (approximately 4 in. diameter and 10 in. length), where they were collected by the EPSS, operated at a sampling flow rate Q_S (10 L/min). After a certain sampling time, t (10, 30 or 60 min), the fungal particles deposited on the collecting electrode were removed by a water droplet rolling under gravity and were collected in a vial. Another stable DC high voltage power supply (Bertan Associates, Inc, Valhalla, NY) provided collecting voltage (V_{EPSS}= -7kV) to the EPSS . The entire experimental setup was housed inside a Class II Biosafety cabinet (NUAIRE Inc., Plymouth, MN).

6.3 Development of bioluminescence method to analyze collected fungi

A choice of a sample analysis technique is one of the major considerations for any bioaerosol detection system as well as when analyzing performance of bioaerosol samplers. Various sample analysis techniques have been used to assess performance of bioaerosol samplers challenged with bacteria: quantitative real-time polymerase chain reaction, acridine orange epifluorescence microscopy, culture-based analysis method, bioluminescence-based technique and others (An et al., 2006; Seshadri et al., 2009; Williams et al., 2001; Zeng et al., 2004).

The culture-based analysis is easy to use, but it is time-consuming, detects only culturable organisms, and organisms may become damaged and non-culturable during sample collection (Buttner and Stetzenbach, 1991; Lin, 2000; Martinez, 1998). The DNA-based analysis has been widely use to detect the presence of biological agents in samples, but its application to analyze performance of bioaerosol samplers is only beginning (An et al., 2006; Han et al., 2009). Our earlier study showed that bioluminescence-based technique which detects the presence of adenosine triphosphate (ATP) could be successfully used to quickly quantify bacteria when analyzing samplers' collection efficiency (Seshadri et al., 2009). The ATP is a basic energy molecule present in all types of living organisms, and thus it is possible to quantify microbial biomass by measuring the ATP content in a sample using bioluminescence. To quantify spores collected by a sampler being tested one needs calibration curves (luminescence intensity as a function of cell concentration) obtained from the study-specific sampler and target biological particles.

6.3.1 Effect of culture age

In the ATP-based detection method, the bioluminescence intensity of a sample is proportional to its ATP content, which, in turn, is proportional to the concentration of biological particles in the sample. When applying this method for fungi, we followed the procedure developed in our previous study for quantification of bacteria (Seshadri et al., 2009). However, since the growth time of fungal cultures may affect the intensity of bioluminescence, the first set of experiments was performed with fungal cultures of different ages: 5, 7, 9, 12, 14, and 21 days to determine the optimum growth time for the method. From 30 mL suspensions containing test fungi, triplicate 200 µL aliquots were extracted and each divided equally between two 1.5 mL

centrifuge tubes: 100 µL for ATP analysis and 100 µL for analysis by acridine orange epifluorescence microscopy (AOEM). For the ATP analysis, the selected volume of fungal suspension (100 µL) was combined with equal volume of Bactiter-Glo reagent (Promega Corp., Madison, WI); the contents were briefly vortexed and then left at room temperature for 9 minutes. The luminescence intensity of the resulting suspension was measured by a luminometer (model 20/20n, Turner Biosystems Inc., Sunnyvale, CA) and recorded as the relative luminescence units (RLU). The luminescence intensity was correlated with concentration of fungal spores in a sample determined by the AOEM as described below. The ratio of luminescence intensity (RLU) normalized to the total number of spores (N_{sample}) was plotted as a function of culture age for each fungal species and is shown in Figure 6.2. The highest ratios for *C. cladosporioides*, *P. melinii*, and *A. versicolor* were 7.7±0.4, 5.9±1.0, and 38.8±7.0, respectively, and they were obtained with 7 day old cultures. Thus, this optimal culturing period of 7 days was chosen for all subsequent experiments.

6.3.2 Preparation of calibration curves

One can also see from Figure 6.2 that different fungal species produce luminescence signal of different strength. Thus, separate calibration curves have to be prepared for each test species. In addition, our earlier research (Seshadri et al., 2009) indicated that the calibration curves should be study-specific, i.e. they should be prepared using the same sampling protocol that will be used to collect actual samples. The calibration curve for each fungal species represents the relationship between the RLU reading from the luminometer and the total spore counts obtained by the AOEM.

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

The calibration curves were obtained by plotting the spore number in 20 µL vs. the RLU values and are shown in Figure 6.3. R^2=0.999 for all three species. The number of spores in a sample (N_{sample}) can then be determined using the equations shown in Figure 6.3:

$$N_{sample} = f(RLU) \tag{6.1}$$

For any given spore concentration, the highest luminescence intensity was observed from *C. cladosporioides* spores and the lowest from *P. melinii* spores. If RLU values for *P. melinii* are set to 1, then intensity ratios for *C. cladosporioides* and *A. versicolor* are approximately 11.0 and 4, respectively. The reliable quantification ranges allowed by the ATP method were $50 \div 5\text{x}10^3$ spores/20 µL for *C. cladosporioides*, $1.6\ \text{x}10^3 \div 1.5\text{x}10^5$ spores/20 µL for *P. melinii*, and $1.3\ \text{x}10^2 \div 1.1\text{x}10^4$ spores/20 µL for *A. versicolor*. Quantification of lower spore concentrations was

unreliable due to the properties of individual species (weak signal) and the background luminosity. All the ATP measurements were performed with water diluents and adjusted for average background values for water (RLU =1500).

When calculating the collection efficiency of the EPSS, the number of collected fungal spores was determined using the corresponding RLU values and equations shown in Figure 6.3. The ATP-based bioluminescence method allows for rapid quantification of spores in a sample, and thus could be a suitable alternative for the microscopic counting method of microorganisms.

6.3.3 Quantification of spores collected by the EPSS

The number of fungal spores collected by the EPSS was determined by both acridine orange-based epifluorescence microscopy (AOEM) (An et al., 2006) using the Axioskop 20 microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY) and the ATP bioluminescence method. The volume of samples collected by the EPSS (10 or 40 μL) was increased to 200 μL by adding sterile purified water and then divided into two equal 100 μL parts: one for the ATP analysis and the other for the AOEM analysis. Each part was placed into a 1.5 mL centrifuge tube.

For the ATP analysis, the first 100 μL aliquot was combined with an equal volume of Bactiter-Glo reagent (Promega Corp., Madison, WI) and luminescence intensity was determined as described above.

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

$$N_{sample} = N \times X \times D_{10-fold} \tag{6.2}$$

Here, N is the average spore count in each microscope view field, X is the number of fields for the entire filter (X=980 for Axioskop 20), and $D_{10\text{-}fold}$ is the dilution factor.

6.4 Determination of the EPSS collection efficiency

The collection efficiency of the EPSS was determined by comparing the number of fungal spores collected by the sampler with the number of spores that would be drawn into the sampler during its collection period (reference concentration). The majority of particles deposited on the collection electrode of the EPSS are removed by the 1st water droplet, as shown in our earlier work (Han et al., 2009; Han and Mainelis, 2008). Thus, the collection efficiency and the concentration rate were calculated based on the 1st water droplet.

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

$$\eta = \frac{N_{sample}}{N_{reference}} = \frac{N_{sample}}{R_{APS} \times 1000 \times Q \times t} \tag{6.3}$$

Here, N_{sample} is the number of spores collected in a water droplet (10 or 40 μL) as determined either by microscopy or ATP the method; $N_{reference}$ is the reference spore number measured by the APS, R_{APS} is the average spore concentration (#/cm^3) measured by the APS every 20 seconds for 10 minute sampling time (every 60 s for 30 min, and every 120 s for 60 min sampling time), Q_s (L/min) is the sampling flow rate of the EPSS, and t (min) is the sampling time. The concentration rate was calculated as follows (Han and Mainelis, 2008):

$$R_C = \frac{Q}{V}\eta, \text{ min}^{-1}, \tag{6.4}$$

Where V is the droplet volume in L.

6.5 Results and discussion

Figure 6.4 shows the representative number concentration and particle size distributions of airborne ,*C. cladosporioides, P. melinii*, and *A. versicolor* spores measured by the APS averaged over 10 min period. The mode diameter for *C. cladosporioides*, *P. melinii*, and *A. versicolor* were approximately 2.0, 2.1, and 1.8 μm, respectively.

Figures 6.5 and 6.6 show the collection efficiency and the resulting concentration rate of the EPSS, respectively, when sampling fungal spores at 10 L/min and using water droplets of 10 and 40 μL to remove the collected particles. The collection efficiency, η, was determined for airborne spore concentrations of approximately 10^2 -10^3 spores/L which, as was determined in the preliminary experiments, could be comfortably counted under a microscope without dilution. The sampling time in these tests was 10 minutes and the number of collected spores was determined by the AOEM and the ATP methods. As could be seen from Figure 6.5, the collection efficiencies of the EPSS measured by the ATP and the AOEM were 21% ± 2.3% and 21% ± 0.4% for *C. cladosporioides*, 36% ± 2.4% and 36% ± 2.0% for *P. melinii* and 25% ± 2.8% and 23% ± 2.7% for *A. versicolor* when using 40 μL collection droplets. For each analysis method and for each spore type, the collection efficiency was lower with the smaller water

droplet (10 μL) compared to the larger droplet (40 μL). The highest decrease, approximately 50%, was observed for *C. cladosporioides*. For the other two fungi the decrease was 20-30%. A similar dependence of the collection efficiency on the size of the collecting droplet was observed when testing with the PSL particles of different sizes. The decrease was attributed to a less efficient removal of particles deposited on the collection electrode by a smaller droplet (Han and Mainelis, 2008). Overall, the collection efficiency for *C. cladosporioides* was lowest among the three species tested. There was a very good agreement in collection efficiencies determined using the AOEM and ATP methods for all test conditions (three types of test particles and two droplet sizes) and differences were not statistically significant ($p>0.05$).

Figure 6.6 shows the concentration rates based on the collection efficiency data presented in Figure 6.5. As could be seen, the concentration rates for *C. cladosporioides*, *P. melinii*, and *A. versicolor* reached approximately $5x10^4$, $9x10^4$, and $8x10^4$, for 40 μL water droplet, respectively, while the concentration rates were for $1x10^5$, $3x10^5$, and $2x10^5$ for 10 μL water droplet, respectively. These concentration rates were lower than values observed for bacteria (~ 0.9 μm) and PSL particles (~2 μm), but still higher than concentration rates of currently available bioaerosol samplers. In addition, these values were observed for a particular reference concentration of airborne spores ($10^2 \div 10^3$ /L). In the ambient environment, spore concentrations can vary widely and in most cases are approximately 10/L, but concentrations as high as 10^4/L could be encountered.

Since the concentration of fungal spores in ambient environment could vary widely, in the next set of experiments we explored the effect of airborne spore concentration ($10^0 \div 10^4$/L) on the collection efficiency of the EPSS and the results are presented in Figure 6.7. Here, the total number of spores collected in a 40 μL droplet is plotted as a function of the reference spore number, $N_{reference}$, determined according Eq. 6.3. The reference spore represents the number of spores entering the sampler during collection period. Since the 40 μL droplet yielded higher collection efficiencies compared to 10 μL droplet, only 40 μL was used. Due to the limited sensitivity of the microscopy at lower concentrations, the sample analysis was performed only using the ATP-based method. The reference spore number was determined using the APS. For each experiment, fresh spores were harvested after 7 days of incubation.

As could be seen from Figure 6.7, at low airborne spores concentrations, ~ 10-100/L ($N_{reference}$ = 10^3-10^4), the number of spores collected in the droplet, N_{sample}, is close to $N_{reference}$, i.e., the collection efficiency of the EPSS is very high. In fact, for the lowest concentrations tested, the collection efficiency exceeded 65% for all fungal species. Thus, for predominant concentrations of ambient airborne fungi (~1-10/L, or 10^3-10^4/m^3), the sampler's collection efficiency and the resulting concentration rate would be substantially higher than those shown in Figures 6.5 and 6.6. On the other hand, as the $N_{reference}$ increases, the N_{sample}, also increases, however, at a lower rate, i.e., the collection efficiency of the EPSS becomes progressively lower. The rate of change however, seems to depend on the fungal species. For the airborne concentration of approximately 10^4/L ($N_{reference} = 10^6$), which was the highest tested, the collection efficiencies for *C. cladosporioides* and *A. versicolor* were under 5%, while the collection efficiency for *P. melinii* was ~20%. Thus, this sampling technology may not best suited for sampling very high concentrations of spores. However, ambient concentrations of fungi of 10^4/L, or 10^7/m^3, are

rarely encountered in ambient environment and the primary application of the EPSS is detection of low bioaerosol concentrations (10^0-10^1/L) due to its high concentration rates.

The results presented in Figure 6.7 could be explained based on the hygroscopicity of the fungal spores. It is generally accepted that fungal spores are hydrophobic (Reponen et al., 2005). Thus, when the collecting ware droplet moves over the spores deposited on the collection electrode, majority of the spores attach to the droplet and do not penetrate into in it. At low numbers of deposited spores, majority of spores can attach to the droplet which results in high collection efficiency. At high numbers of deposited spores, the surface of the water droplet quickly becomes saturated and cannot accept any more fungal spores. As a result, the collection efficiency of the EPSS substantially decreases for higher spore concentrations. Theoretically, 10^6 to $5x10^6$ fungal spores with diameter 2-3 μm could be uniformly packed onto the surface of a 40 μL droplet, while approximately 10^9 spores would fit inside such a droplet. The highest number of spores we were able to collect was in the order of 10^5-10^6 spores in a 40 μL droplet. Thus, at high reference concentrations we were approaching saturation limit of the droplet, i.e., many of the spores deposited on the collection electrode were not picked up by the droplet.

Naturally, not all spores will accumulate only on the droplet's surface, but some will penetrate into the droplet. The ratio of spores on the droplet vs. spores inside the droplet is most likely determined by the hydrophobic properties of each individual spore type. As could be inferred from Figure 6.7, the maximum number of spores that could be accommodated by a droplet is different for each spore type and likely represents difference in their hydrophobicity. Based on this reasoning, the *C. cladosporioides* spores seem to be most hydrophobic, while the *P. melinii* spores seem to be least hydrophobic of the three tested species. The data presented in Figure 6.5 also show that the highest collection efficiency was observed for *P. melinii* spores and the lowest for *C. cladosporioides* spores.

The tests described earlier were performed with 10 min sampling time. For many sampling projects, longer sampling times are needed and thus additional tests were performed for 30 and 60 min sampling time. Given the data presented in Figure 6.7, i.e., decrease of the collection efficiency at higher spore loadings, the airborne spore concentration was adjusted so that the total number of spores entering the EPSS would be approximately the same for all three sampling times. The collection efficiency of the EPSS tested at 10 L/min with the 40 μL droplet for 10, 30 and 60 min sampling time is presented in Figure 6.8. The collected spores were quantified by both the epifluorescence microscopy (AOEM) and the ATP-based method. When the sampling time was increased from 10 minutes to 60 minutes, the EPSS collection efficiency for *C. cladosporioides* analyzed by the AOEM decreased from 21% ± 4% (21 ± 0.4% by ATP) to 6% ± 0.8% (6 ± 0.7% by ATP). The decrease for the other two fungi was much less pronounced: for *P. melinii* ,from 36% ± 2.4% (36 ± 2.4% by ATP) to 31% ± 5.7% (31 ± 3.9% by ATP); for *A. versicolor,* from 25% ± 2.8% (23 ± 2.7% by ATP) to 20% ± 1.8% (19 ± 1.6% by ATP). The decrease in the collection efficiency with longer sampling time was also observed in our earlier studies with bacteria (Han et al., 2009). The decrease could be explained by a less efficient removal of the deposited particles by the water droplet due to the increase in the adhesive forces between the particles and the collection surface with prolonged contact time (Hinds, 1999). The increase in the adhesion a force seem to depend on the particle type and in our case seems to have been most pronounced for *C. cladosporioides*. Even though the average

collection efficiency for three fungi after 60 min sampling time was approximately 20%, one has to keep in mind that all particles removed from the EPSS are concentrated in only one droplet. Therefore, the entire sample could be analyzed thus increasing about ability to detect the particle presence. As could be observed from Figure 6.8, the average collection efficiency determined using the two methods (AOEM versus ATP) was in a very good agreement: the difference between the two methods was not statistically significant ($p>0.05$) for all three spores and all three sampling times.

6.6 Figures for Chapter 6

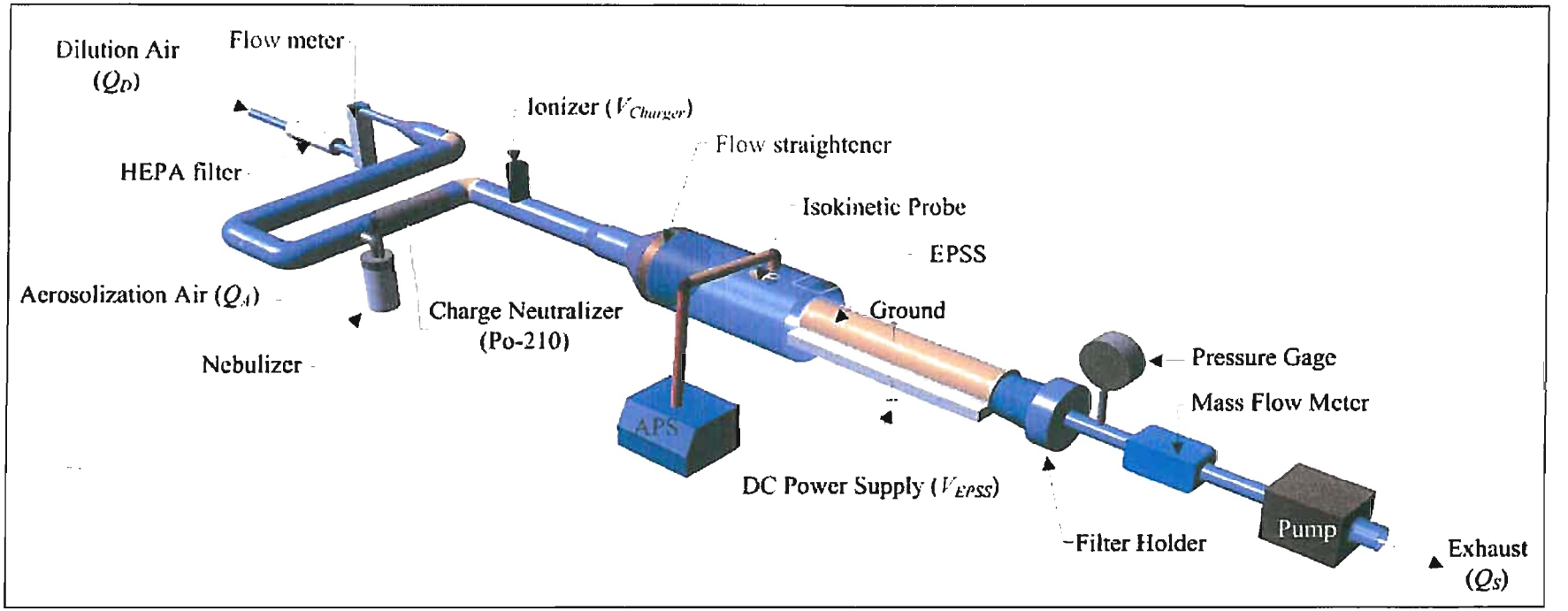

Figure 6.1. Schematic diagram of the experimental setup. The figure in the upstream of the EPSS shows an isokinetic probe used to determine the bioaerosol reference concentration.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

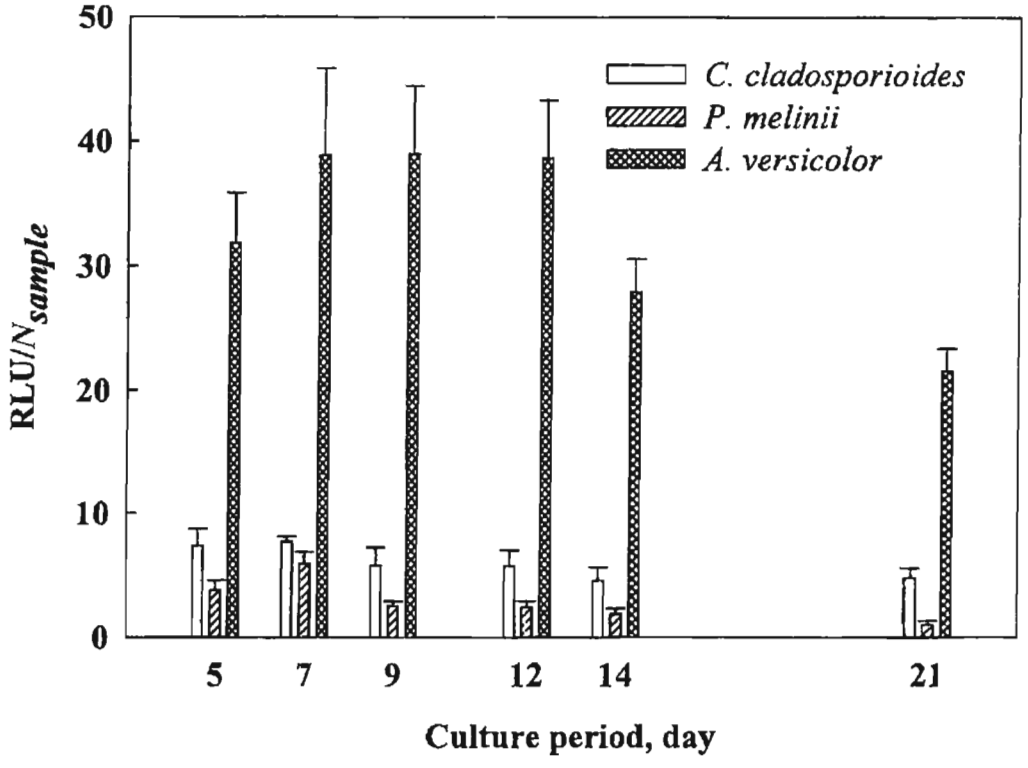

Figure 6.2. Ratio of the luminescence intensity (RLU reading) normalized to total spore number as a function of culture age (5, 7, 9, 12, 14, 21 days) for three test fungi. The total spore number was determined by Acridine Orange Epifluorescence Microscopy (AOEM). The error bars represent the standard deviation from three repeats.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

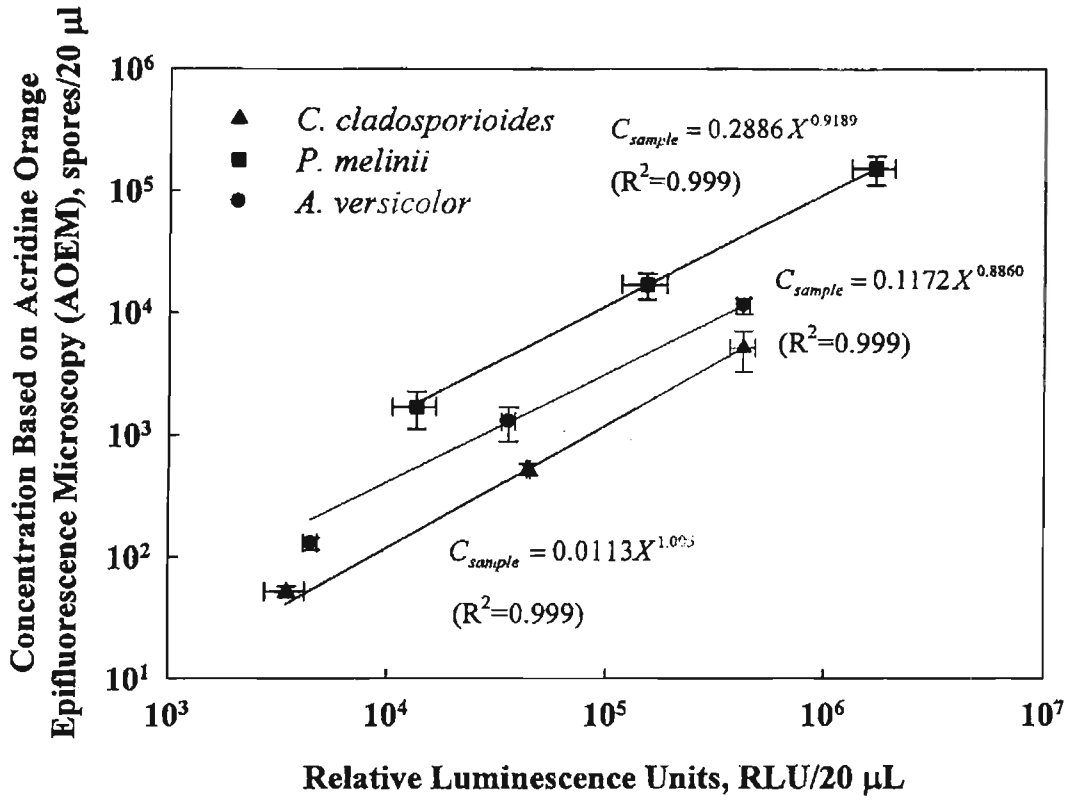

Figure 6.3. Calibration curves for *C. cladosporioides, P. melinii, and A. versicolor* spores based on the relationship between the RLU values and the fungi concentration as determined by the epifluorescence microscopy.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

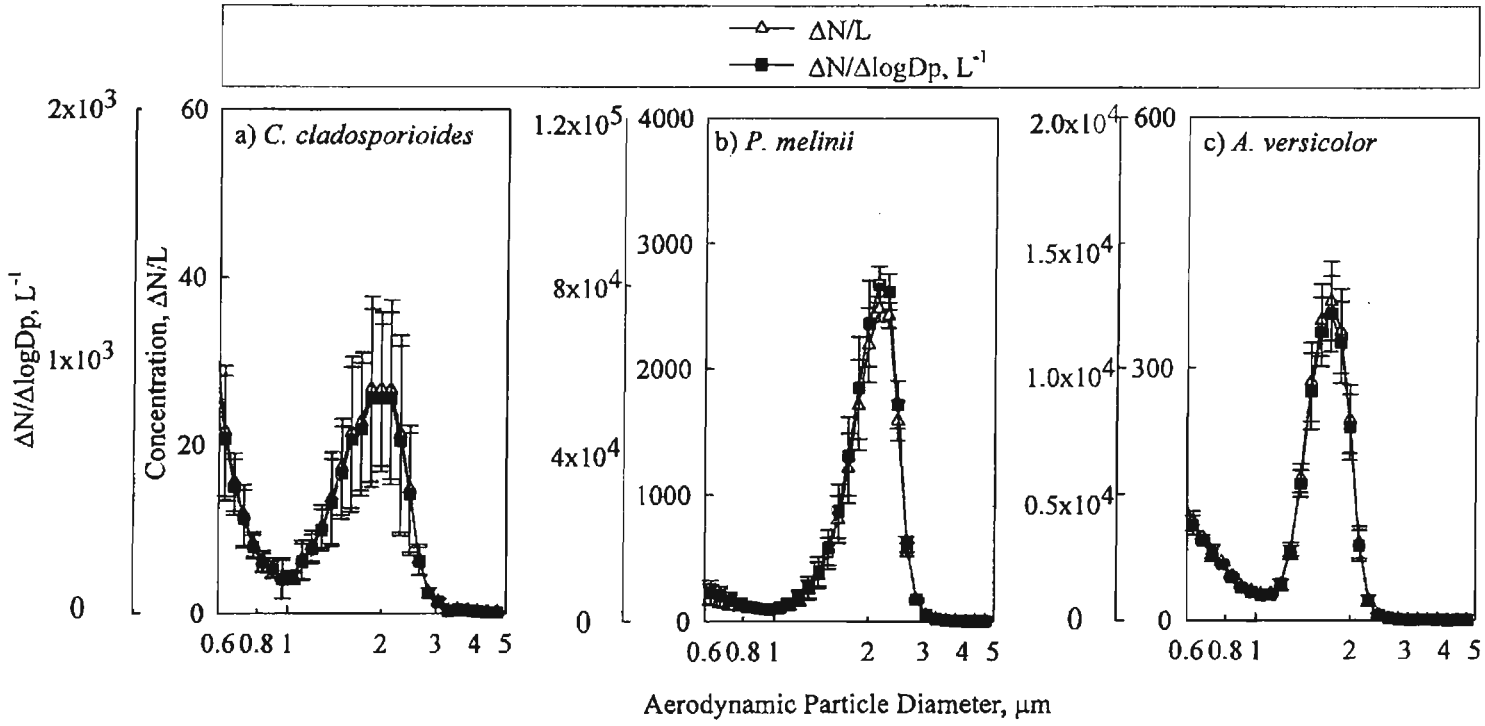

Figure 6.4. Particle size distributions according to number concentration (ΔN/L) and normalized number concentration (ΔN/ΔlogDp, L^{-1}) for three test fungi: (a) *C. cladosporioides*, (b) *P. melinii*, and (c) *A. versicolor*..

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

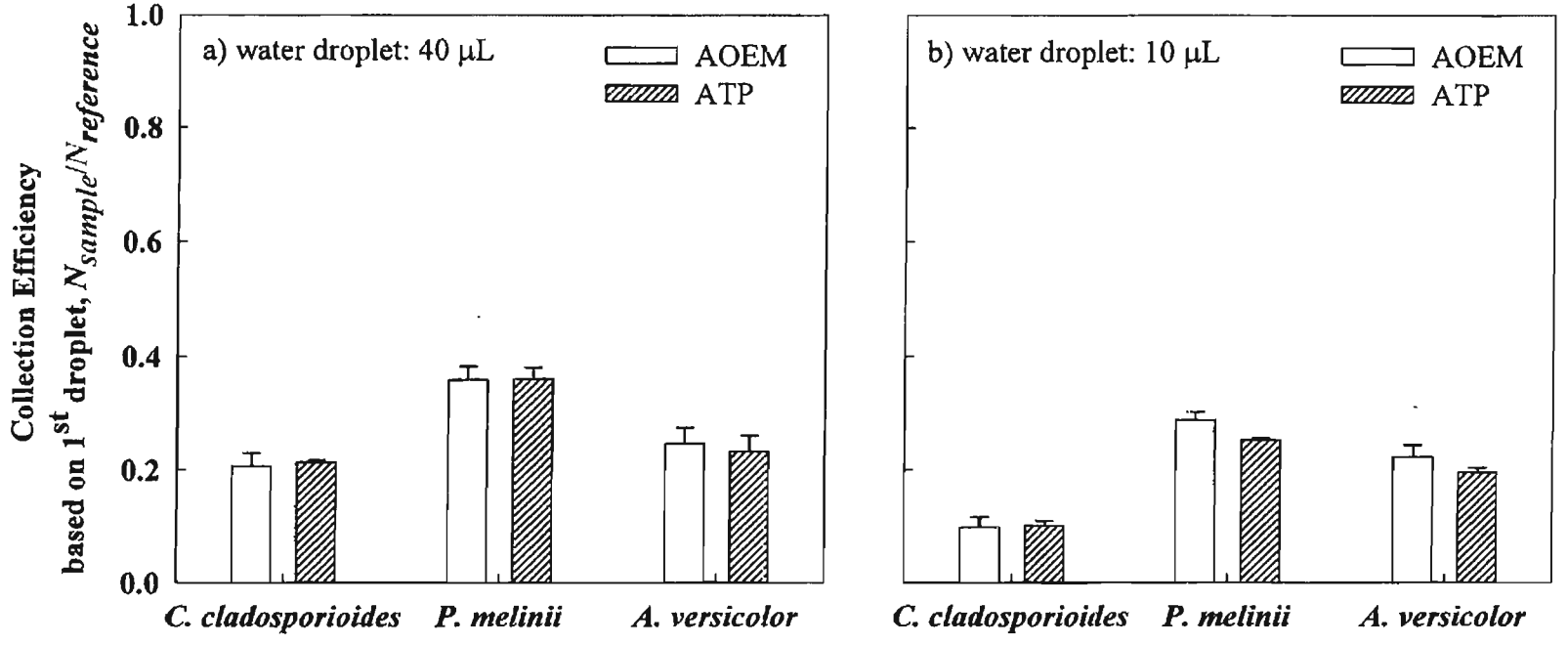

Figure 6.5. Collection efficiency of the EPSS when collecting spores of *C. cladosporioides, P. melinii, and A. versicolor.* The eficiency is based based on the 1st water droplet (40 and 10 μL) at 10 L/min sampling flow rate, the 12 V/50 mA charging condition and 7 kV collections voltage. The error bars represent the standard deviation from three repeats.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

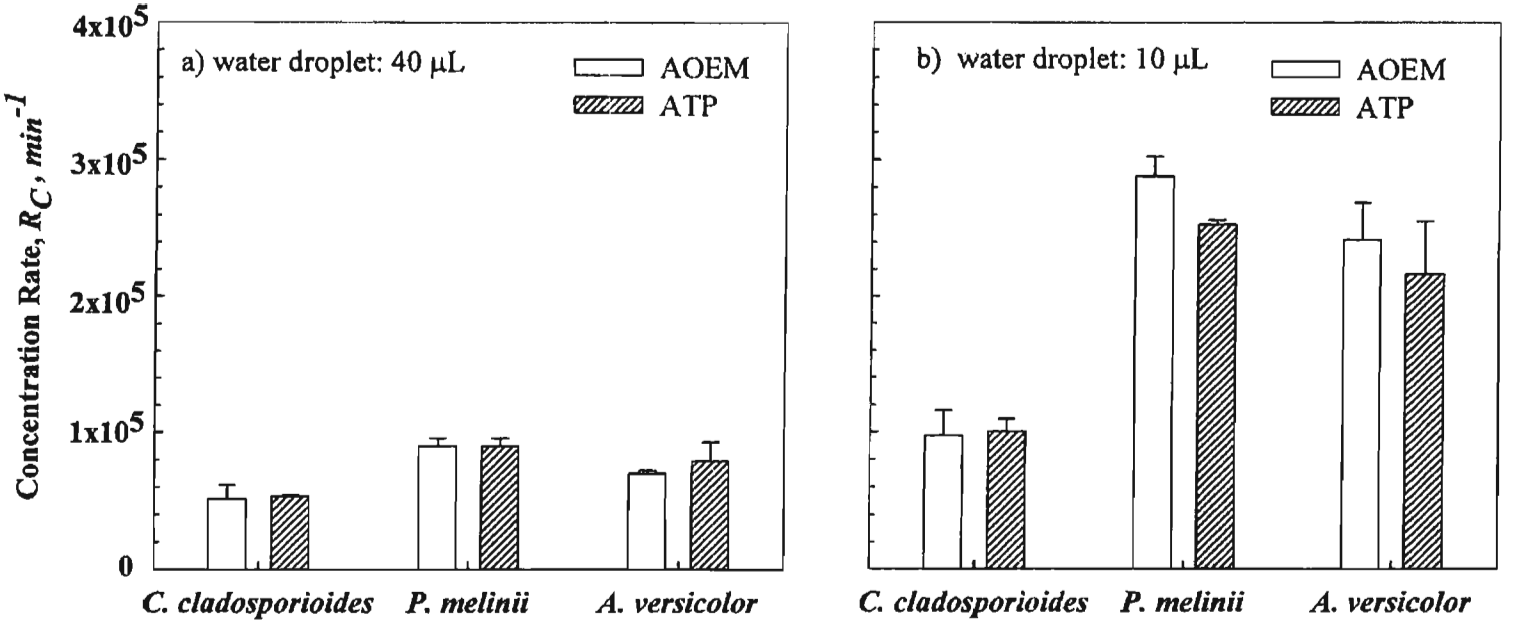

Figure 6.6. Collection rate of the EPSS when collecting spores of *C. cladosporioides, P. melinii, and A. versicolor.* The concentratoin rate is based based on the 1st water droplet (40 and 10 μL) at 10 L/min sampling flow rate, the 12 V/50 mA charging condition and 7 kV collections voltage. The error bars represent the standard deviation from three repeats.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

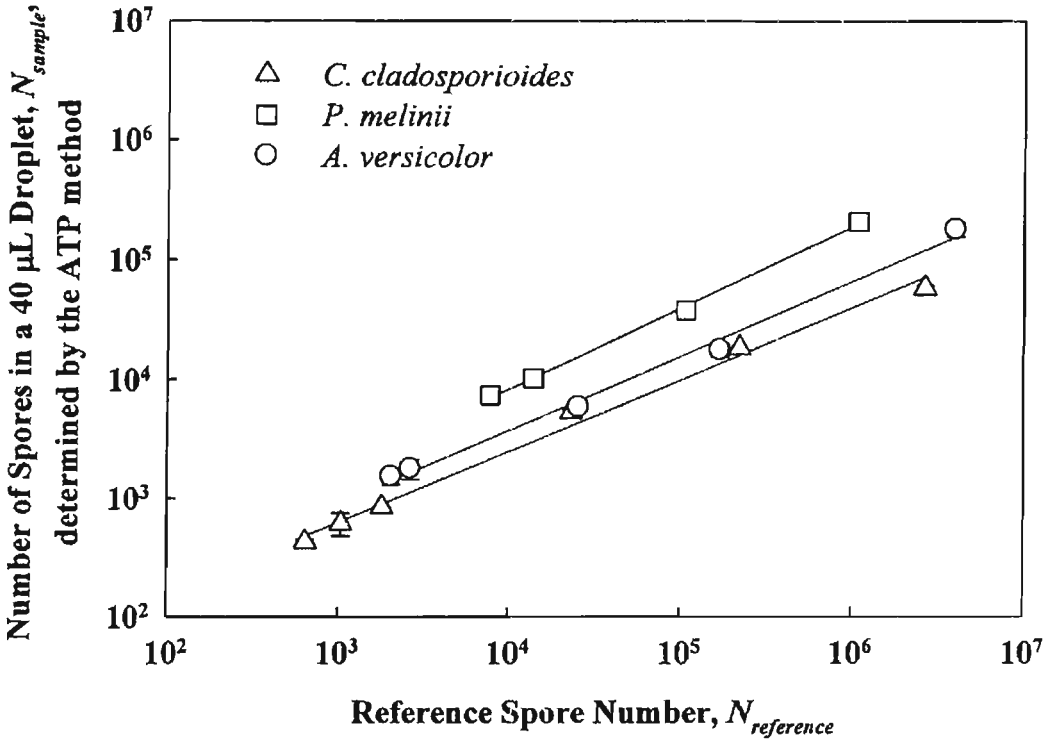

Figure 6.7. Total spore counts based on the 1st water droplet (40 μL) as a function of the reference spore number at 10 L/min sampling flow rate, 12 V/50 mA charging condition and 7kV collection voltage for spores of *C. cladosporioides, P. melinii, and A. versicolor*. The error bars represent the standard deviation from three repeats.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

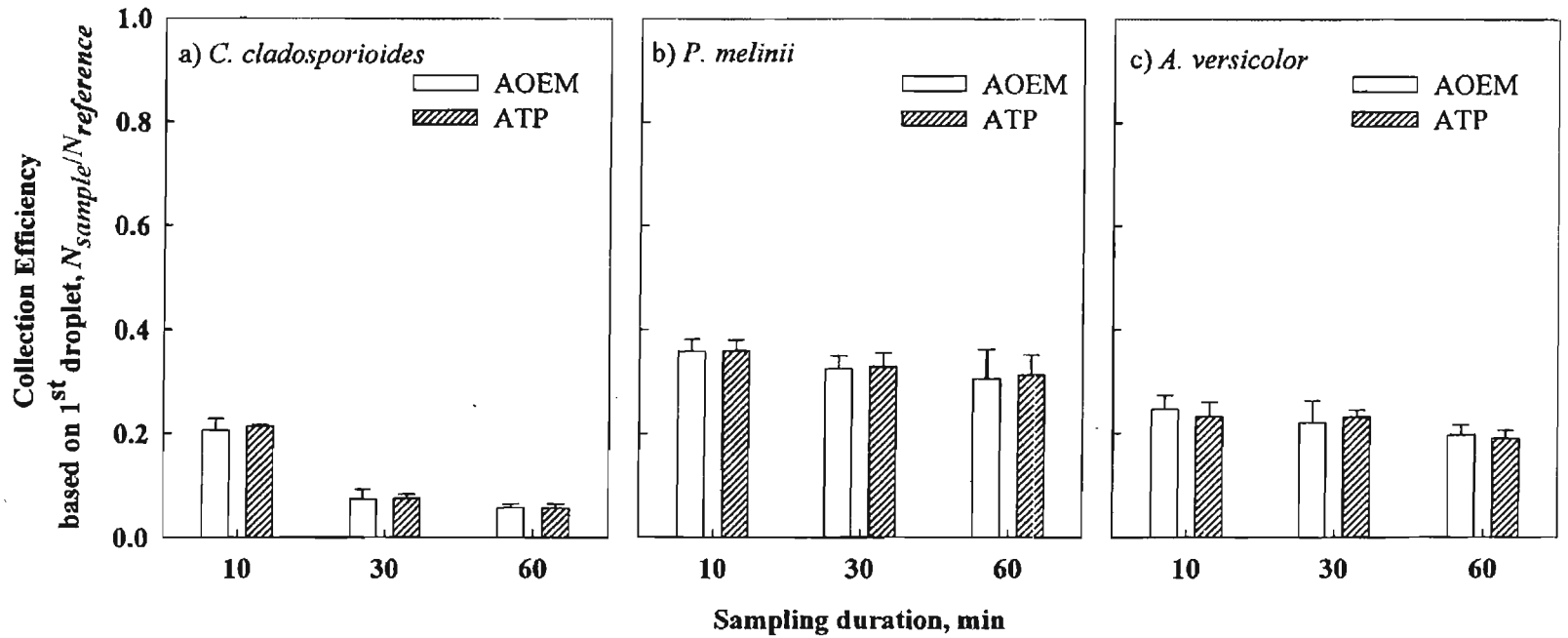

Figure 6.8. Collection efficiency of the EPSS based on the 1st water droplet (40 μL) as a function of sampling time (10, 30, and 60 min) at 10 L/min flow rate, 12 V/50 mA charging condition and 7kV collection voltage for spores of *C. cladosporioides, P. melinii, and A. versicolor*. The error bars represent the standard deviation from three repeats.

REF: Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

7 *Comparison of the electrostatic sampler against another sampler*

The research described in this chapter responds to the Specific Aim VI "Laboratory evaluation of the EPSS against other bioaerosol samplers" and the Specific Aim VI "Preliminary field evaluation of the EPSS against other bioaerosol samplers". The main goal of the entire research project was to evaluate the feasibility of a novel bioaerosol sampler where electrostatic collection technique is combined with superhydrophobic collection surface. This was accomplished by designing, building and evaluating such a sampler prototype. As described in the chapters above, our efforts were successful and a bench top model achieved unprecedented sample concentration rates exceeding 10^6/min when challenged with airborne polystyrene latex particles, different bacteria and fungi. The building of the sampler, determining its optimum operating conditions, and testing it with said particles consumed the vast majority of our effort. Therefore, only a limited electrostatic sampler's comparison against other samplers was performed. The comparison included side-by-side operation with a BioSampler (SKC Inc., Eighty Four, PA) under laboratory and field conditions. A more extensive investigation of the electrostatic sampler's performance in the field will be performed in future studies, where a field-deployable sampler will be constructed.

7.1 Sampling of fungi in laboratory environment

The Electrostatic Precipitator with Superhydrophobic Surface (EPSS) and a BioSampler (SKC Inc., Eighty Four, PA) were operated in parallel in the controlled laboratory environment. The experimental setup shown in Figure 6.1 was used, and both samplers collected fungi (*Cladosporium cladosporioides, Penicillium melinii*, and *Aspergillus versicolor*) simultaneously from the test chamber. The fungi preparation procedures were the same as described in Chapter 6. Both samplers operated at 10 L/min and the sampling time was 10 min. The volume of the EPSS collection droplet was 40 μL. The nominal BioSampler's collection flow rate is 12.5 L/min, however our earlier research showed that it operates just as efficiently, if not more effectively, at 10 L/min (Seshadri et al., 2009). The BioSampler was operated with 5 mL of collection fluid. The reference spore concentration was provided by an Aerodynamic Particle Sizer (APS 3321, TSI, Inc.). Upon finishing the collection, the contents of both samplers were analyzed using the ATP-based bioluminescence technique as described in Chapter 6. For the BioSampler, the 40 μL was analyzed and the determined spore concentration was translated for the entire sample volume (~4 mL) remaining after 10 min sampling time. Due to a relatively large sample volume of the BioSampler, the concentration of airborne fungi had to be in the range of 10^2-10^3 spores/L. At lower airborne concentrations, the spores collected by the BioSampler were not within the detection range of the ATP method.

Figure 7.1 shows the collection efficiency of the two samplers, while Figure 7.2 shows the corresponding concentration rate. Both values were determined as described in chapters above. As could be seen from Figure 7.1, the EPSS had collection efficiency in the range of 20-40%, while the BioSampler had the collection efficiency of 60-70% depending on the spore type. However, the concentration rates of the two samplers are vastly different as shown in Figure 7.2 due to the EPSS' ability to concentrate fungal spores in very small amount of liquid. The

concentration rate of the EPSS ranged from $5x10^4$ to almost $1x10^5$, while the concentration rate of the BioSampler was approximately $1.1x10^3$/min. Thus, for the applications where only a small sample amount can be analyzed, the EPSS is advantageous.

7.2 Sampling of biological particles in the indoor environment

In this part of the study, we compared performance of the EPSS and BioSampler when sampling particles in an occupational indoor environment. Since the room was continuously ventilated and the air was filtered as part of the normal HVAC system's operation, the expected microorganism concentration was low and the environment was suitable to check the performance of the EPSS at low particle concentrations. Both the EPSS and BioSampler were operated at 10 L/min and the sampling time was 15 min for each test. The BioSampler was operated with 20 mL of sterile deionized water. The EPSS drew the particles in through a 0.035 m duct, which housed the particle charging unit. The charging unit operated at 12V/50mA. The particles collected by the EPSS were removed by 40 μL droplets. Samples collected by both devices were analyzed using the ATP method as described above and 40 μL of sampling liquid was used from each sampler. The readings were recorded as relative luminescence units (RLUs), adjusted for background RLU values and the data are shown in Figure 7.3. The RLU readings for the EPSS ranged from approximately 1500 to 2500. Since the airborne bioaerosol concentration was low, the BioSampler readings were very close to the background and were below limit of quantification (LOQ).

Using the data from Figure 7.3 we attempted to estimate concentration of airborne biological particles. Since we likely encountered different microorganisms, including both bacteria and fungi, we used a combination of calibration curves: three from fungi as described in Chapter 6 and two from bacteria as described earlier (Seshadri et al., 2009). By using an average microorganism concentration provided by each curve and the total volume of air sampled (0.15 m^3) we estimated total concentration of viable airborne microorganisms and the data are presented in Figure 7.4. As could be seen, the total bioaerosol concentration ranged from 4,000 to 7,000 biological particles per m^3. We would like to stress that this is only an estimate based on a combination of five available calibration curves. It is meant to provide only an approximate estimate of airborne microorganism concentration. Common indoor office concentrations of culturable bacteria and fungi and usually in the 500-1000 CFU/m^3 rage. Since the ATP-based bioluminescence detects viable particles (culturable particles are a fraction of viable particles) and the technique detects both bacteria and fungi together, the obtained bioaerosol concentration is within expected range. For the BioSampler, the concentration was too low to be detected with the ATP method.

The data presented in Figures 73-7.4 indicate that the EPSS allows determining low airborne microorganism concentrations.

7.3 Figures for Chapter 7

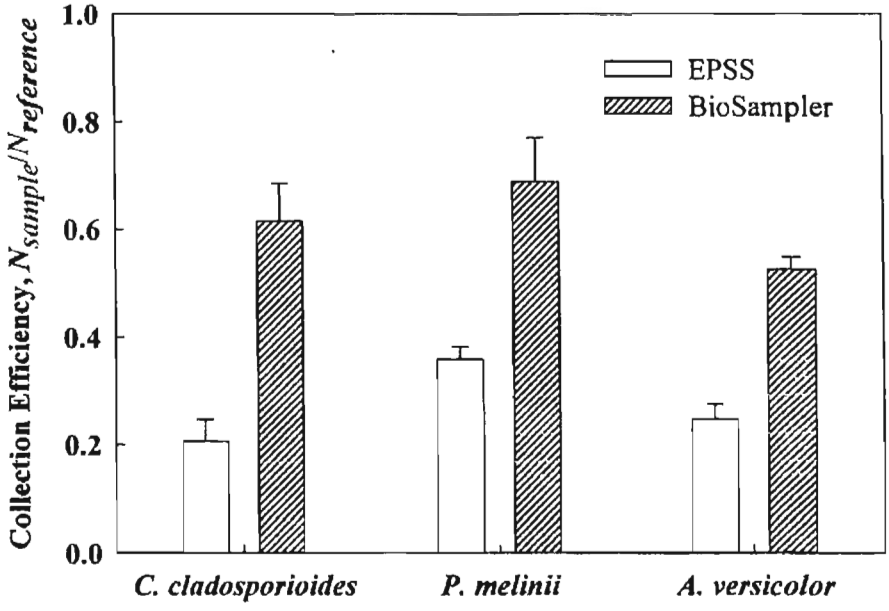

Figure 7.1. Comparison of the collection efficiency between the EPSS and the BioSampler when sampling *C. cladosporioides*, *P. melinii*, and *A. versicolor* fungal spores. Both samplers operated at 10 L/min sampling flow rates; the EPSS operated at 12 V/50 mA charging condition and 7kV collection voltage. The error bars represent the standard deviation from three repeats.

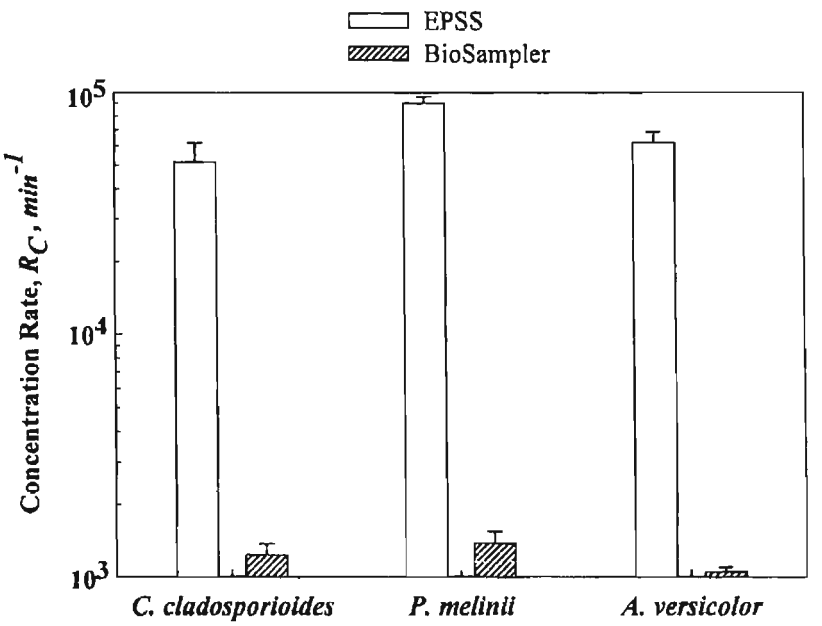

Figure 7.2. Comparison of concentration rates of the EPSS and the BioSampler when sampling fungal spores of *C. cladosporioides*,*P. melinii*, and *A. versicolor* at 10 L/min. Performance of hte EPSS is based on the 1st 40 μL water droplet and 10 L/min flow rates and at the 12 V/50 mA charging condition and 7 kV collection voltage. The error bars represent the standard deviation from three repeats.

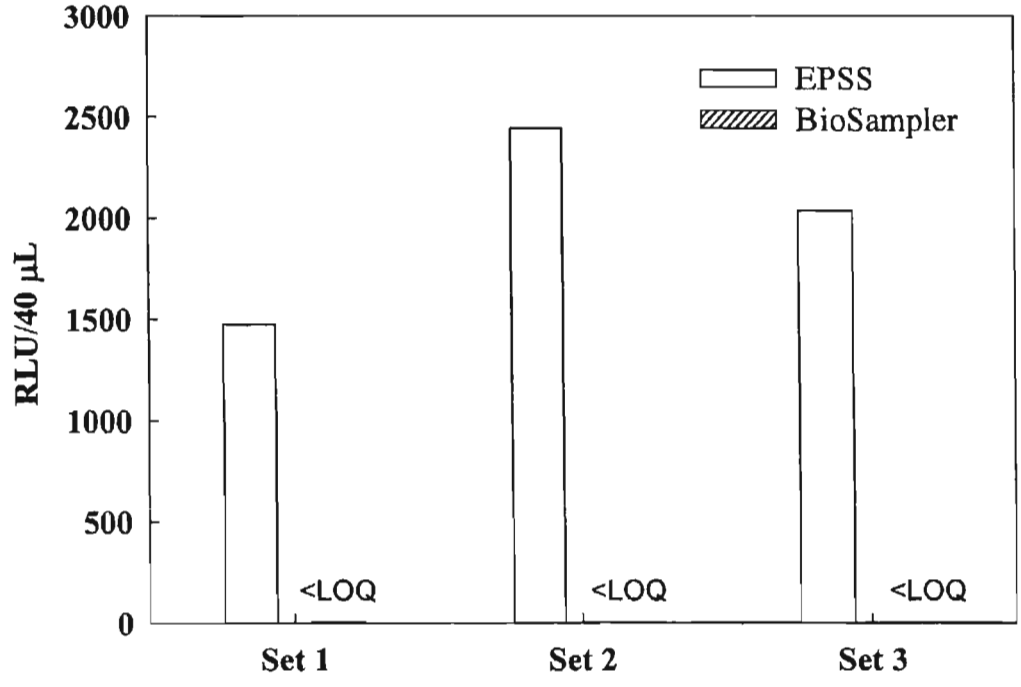

Figure 7.3. Comparison of RLU values between the EPSS and the BioSampler at 10 L/min flow rates for both samplers when sampling in an occupational indoor environment. The samples were analyzed using ATP-based bioluminescence. All values are adjusted for background values.

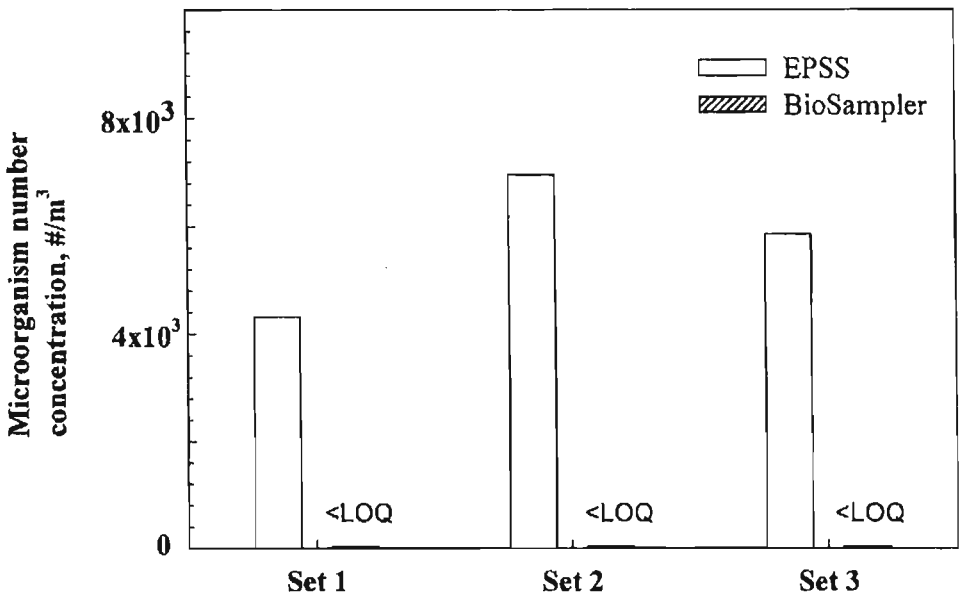

Figure 7.4. Comparison of airborne microorganism concentration determined by the EPSS and the BioSampler in an indoor occupational environment. Both samplers operated at 10 L/min sampling flow rates The samples were analyzed using ATP-based bioluminescence. All values are adjusted for background values.

8 Summary, conclusions and future directions

The main objective of this exploratory research was to improve our ability to measure exposures to airborne microorganisms, especially to their low concentrations, by designing, constructing and evaluating a novel bioaerosol sampler with very high sample concentration rate. Based on the proposed combination of the electrostatic collection method and the use of superhydrophobic surface, a novel bioaerosol sampler with a capacity to achieve concentration rates of over 10^6/min and to accrue collected particles in liquid volumes as small as 5 μL, has been successfully designed and tested with non-biological and biological particles (different bacteria and fungi). The Electrostatic Precipitator with Superhydrophobic Surface (EPSS) achieves this performance using electrostatic precipitation and special conditioning of the collection electrode. Inspired by the Lotus leaf, application of the superhydrophobic coating on the collection surface provides for highly efficient transfer of deposited particles into a liquid sample.

The prototype EPSS has a shape of a closed half-pipe, where a top plate serves as the ground electrode, while the collecting surface is a 3.2 mm wide rectangular electrode coated with a superhydrophobic substance and positioned in a groove in the flat bottom surface opposite the ground electrode. Airborne particles drawn into the sampler are positively charged and then by the action of an electrostatic field deposited onto the negatively charged electrode. The sampler was positioned at a ~5 degree inclination angle to the horizontal, and the injected water droplets rolled-off of electrode's surface removing deposited particles. Sampler's performance was initially analyzed with non-biological polystyrene latex particles of five aerodynamic diameters (0.5, 1.2, 1.9, 3.2, and 5.1μm), collecting droplet volumes ranging from 5 to 60 μL, and sampling flow rates of 2, 5, and 10 L/min. It was determined that the vast majority of particles deposited onto the electrode are removed by the first rolling droplet, which for 3.2 μm particle and 20 μL droplet translated into a concentration rate of $3x10^5$. By narrowing the electrode to 2.1 mm and lowering the droplet volume to 5 μL we achieved the concentration rate as high as $1.2x10^6$. These concentration rates were sustained for sampling times as long as 60 min.

The use of non-biological polystyrene particles allowed us to determine the optimal parameters for the sampler's operation and a series of tests were performed with biological particles. Experiments with the two common test microorganisms (*Pseudomonas fluorescens* and *Bacillus subtilis*) have shown that the novel bioaerosol sampler with superhydrophobic collection surface can efficiently collect and concentrate airborne bacteria in small amounts of liquid (5 or 40 μL). For 10 min sampling, the collection efficiencies for both bacteria ranged from 50 to 72% and were substantially higher compared to the collection efficiencies for PSL particles of similar size. The difference was attributed to the different nature of particles – biological vs. non-biological – and their differences in accepting externally imparted electrical charge. When used with 5 μL collection droplet and 10 L/min sampling flow rate, this new sampling concept allowed achieving bioaerosol sample concentration rates of more than 10^6/min. One potential drawback of the method was the decrease in the collection efficiency with longer sampling times. Even then, a 60 min sample concentrated in one small droplet would allow obtaining information about the presence of microorganisms at low concentrations. In the ambient environment, the bacteria are often carried by larger particles and the decrease in collection efficiency might be minimal or non-existent as was observed with PSL particles.

Since the primary application of the novel sampler is its use to detect low microorganism concentration, it needs to be compatible with modern sample analysis techniques, such as quantitative polymerase chain reaction (QPCR). As a part of our effort to improve the ability to quantify bioaerosol samples using such techniques, we investigated application of the whole-cell QPCR. In this method, DNA is not extracted from bacteria to the PCR reaction, but the whole cells are used as the reaction template. The whole-cell QPCR method offers certain advantages over conventional QPCR and is simpler and easier to use compared to the traditional QPCR. It is also labor and time-efficient compared to microscopy and allows processing more samples over the same time period, while being suitable to measure particle concentrations that are too low to determine using microscopy. We developed calibration curves for this method and applied it to analyze samples collected by the EPSS as well as investigate its collection efficiency and concentration rate. The collection efficiency of the EPSS determined by the epifluorescence microscopy and the whole-cell QPCR was not statistically different. The sampler's concentration rate averaged for both microorganisms and both methods was ~$1.2x10^6$/min. Thus, this method was shown to be compatible with the EPSS and could be applied when quantifying bacteria collected by the EPSS.

In the next series of tests we analyzed performance of the EPSS when collecting airborne fungi (*Cladosporium cladosporioides*, *Penicillium melinii*, and *Aspergillus versicolor*) with 10 or 40 μL droplets. For airborne spore concentrations of 10^2-10^3/L, and when sampling with 10 μL droplet, concentration rates in the range of $1x10^5$ to $3x10^5$/min were achieved. Although lower than concentration rates observed with bacteria, such concentration rates are higher than those of most currently available bioaerosol samplers, thus improving our detection abilities to detect low concentrations of airborne fungi. In addition, it was demonstrated that the collection efficiency of the EPSS substantially increases at lower spore loadings. For fungal concentrations usually encountered in the ambient environment (10^0-10^1/L), the concentration rate of the EPSS would approach 10^6/min and higher. In addition, we successfully developed and applied an ATP-based bioluminescence method to quantify the collected fungal spores. The spore concentrations determined by the microscopy and the ATP-based method were in very good agreement. In fact, the ATP-based method allowed us to analyze spore concentrations that were too low to reliable detect by microscopy. Thus, the ATP method could serve as a fast and sensitive alternative to traditional microscopy when testing samplers' performance with fungal spores.

In summary, the novel bioaerosol sampling methodology developed during this project provides very high concentration rates with low power expenditure, which is desirable for wide-spread application of the method. In addition, in bioaerosol detection applications, where the agents are generally present is small concentrations, the EPSS would provide a highly concentrated sample is a small liquid volume which can significantly enhance our ability to detect the biological agents. Also, it was shown that the use of liquid sampling medium allows for sample analysis by several methods, including the polymerase chain reaction and ATP-based bioluminescence.

The prototype sampler had the following main characteristics:

- Collection efficiency of ~50-70% and the resulting unprecedented concentration rate of $1.2x10^6$/min (!) when sampling bacteria at 10 L/min with 5 μL droplet.

- Concentration rate of up to $3x10^5$/min when sampling fungal spores at 10 L/min with 10 μL droplet. The concentration rate increases to ~10^6/min, when sampler is challenged with lower airborne fungal concentration (10^0-10^1/L).
- In most cases, a vast majority (>90%) of particles deposited on the collection electrode was removed by the first rolling droplet.
- Compatible with multiple sample analysis methods, including microscopy, quantitative real-time PCR (QPCR) and adenosine triphosphate (ATP) – based luminescence.
- The new sampling concept is feasible and upon its further development will provide a novel tool to estimate exposures to low bioaerosol concentrations. The sampler has small footprint and negligible pressure drop (low power requirement).

One has to keep in mind that this project demonstrated feasibility of the proposed bioaerosol collection method and that further development and exhaustive field testing is needed before this sampling method is deployed in the field. In addition to some very positive results that were achieved, certain critical issues, such as the need for a reliable particle charging unit, became apparent. The experiments were carried out with commercially available ionizers (AS150, Wein Products Inc., Los Angeles, CA), which are among the most popular compact ionizers, and allowed us to show that the proposed sampler concept is feasible and even allowed to achieve unprecedented sample concentration rates exceeding $1x10^6$/min. At the same time a number of shortcomings of such ionizers were discovered. Due to their fixed size and geometry (as would be the case with any other commercially available ionizer), the ionizer was difficult to integrate with the precipitator without substantially obstructing the air flow and the precipitator's operation. We also found out that the ionizer's charging efficiency is relatively low while the losses could be substantial, especially when it is used as designed. In addition, the charger was not able to provide sufficient amount of charge for flow rates above 10 L/min. To improve the proposed sampling technology and to allow it efficiently operate at flow rates above 10 L/min, thus further improving the new sampler's detection limit, a new and more efficient particle charger needs be designed, tested and integrated with the sampler. The new charger should not only efficiently charge the particles and have low losses, but should also be easy to integrate with the new sampler. A development and integration of a new and efficient charging unit with the EPSS would be one of the foci of future studies. Once the charger is developed, all components of the new sampling system (charging unit, collection unit, power sources, air mover, liquid injection and droplet capture system) need to be integrated into a field-deployable system. The system will then have to be exhaustively tested in the laboratory and field environments. Upon successful testing the new sampler could be deployed in various occupational environments. The development of a field-deployable unit is the focus of a submitted R01 grant application.

Publications

Peer-reviewed publications

1. Han, T and Mainelis, G: [2008]. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. Journal of Aerosol Science 39:1066–1078.

This publication responds to Specific Aim I "Design and construction of Electrostatic Precipitator with Superhydrophobic Surface (EPSS)", Specific Aim II "Analysis of the sampler's performance when collecting non-biological particles" and Specific Aim III "Evaluation of the particle removal efficiency from the superhydrophobic surface as a function of droplet size and quantity"

2. An, HR, Mainelis, G and White, L: [2006]. Development and Calibration of Real-time PCR for Quantification of Airborne Microorganisms in Air Samples. Atmospheric Environment 40:7924-7939.
3. An, HR, Han, I-K, White, L and Mainelis, G: [2009]. Quantitative Real-Time PCR for Bioaerosol Detection: Analysis of Factors Affecting Standard Curves. Journal of Aerosol Science: To be submitted.
4. Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria Aerosol Sci. Technol.: Submitted.

These publications respond to Specific Aim IV "Analysis of physical collection efficiency using biological test particles" and specifically with analysis of sampler's performance when sampling airborne bacteria, including "Development and application of whole-cell QPCR techniques to quantify collected bacteria" (ADDED Specific Aim).

5. Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

This publication responds to Specific Aim IV "Analysis of physical collection efficiency using biological test particles" and specifically with analysis of sampler's performance when sampling airborne fungi, including "Development and application of ATP-based bioluminescence technique to quantify collected fungi" (ADDED Specific Aim).

Thesis

An, HR: [2009]. Development and application of quantitative bioaerosol analysis method using PCR [Ph.D. thesis]. New Brunswick: Rutgers, The State University of New Jersey. 159 p.

Proceedings

1. Han, T., and Mainelis, G. [2009] Collection Efficiencies of an Electrostatic Sampler with Superhydrophobic Surface for Fungal Bioaerosols, *Abstracts of the* 28th *Annual Meeting of the American Association for Aerosol Research* (Minneapolis, MN, October 26-30, 2009), 6A5.

2. Han, T., An, H.R, Lioy, PJ, and Mainelis, G. [2008] Advanced Collector for Airborne Bioagents, Abstracts of the *Statewide Homeland Security Research Symposium*, Princeton University (Princeton, New Jersey, December 5, 2008).

3. Han, T, An, H.R. and Mainelis, G. [2008] A Performance of an electrostatic sampler with superhydrophobic surface when collecting bacterial aerosols, *Abstracts of the* 27th *Annual Meeting of the American Association for Aerosol Research* (Orlando, Florida, October 20-24, 2008), 1C.04.

4. Han, T., An, H.R. and Mainelis, G. [2008] A Novel Electrostatic Bioaerosol Sampler with High Concentration Rate, Abstracts of the 2008 Scientific Conference on Obscuration and Aerosol Research, (Battelle Eastern Science and Technology Center, Aberdeen, Maryland, June 26, 2008).

5. Mainelis, G., Han, T.H., Yao, M. [2008] Development of Advanced Techniques for the Collection of Airborne Microorganisms, *Abstracts of the 7th International Symposium on Advanced Environmental Monitoring*, (Honolulu, Hawaii, February 23-28, 2008), I-G04.

6. Mainelis, G. and Han, T.H [2007] Design and Development of an Electrostatic Sampler for Biological Aerosols with High Concentrating Rate, *Abstracts of the* 26th *Annual Meeting of the American Association for Aerosol Research* (Reno, Nevada, September 24-28, 2007), 3B.6.

Inclusion of gender and minority study subjects

NA

Inclusion of children

NA

Materials available for other investigators

Whole-cell QPCR

Since the primary application of the novel sampler is its use to detect low microorganism concentration, it needs to be compatible with modern sample analysis techniques, such as quantitative polymerase chain reaction (QPCR). As a part of our effort to improve the ability to quantify bioaerosol samples using such techniques we investigated application of the whole-cell QPCR. In this method, DNA is not extracted extraction prior to the PCR reaction, but the whole cells are used as the reaction template. The whole-cell QPCR method offers certain advantages over conventional QPCR and is simpler and easier to use compared to the traditional QPCR. It is also labor and time-efficient compared to microscopy and allows processing more samples over the same time period, while being suitable to measure particle concentrations that are too low to determine using microscopy. We developed calibration curves for this method and applied it to analyze the samples collected by the EPSS as well as investigate its collection efficiency and concentration rate.

The method is described in detail in the following publications:

1. An, HR, Mainelis, G and White, L: [2006]. Development and Calibration of Real-time PCR for Quantification of Airborne Microorganisms in Air Samples. Atmospheric Environment 40:7924-7939.
2. An, HR, Han, I-K, White, L and Mainelis, G: [2009]. Quantitative Real-Time PCR for Bioaerosol Detection: Analysis of Factors Affecting Standard Curves. Journal of Aerosol Science: To be submitted.
3. Han, T, An, HR and Mainelis, G: [2009]. Performance of an Electrostatic Precipitator with Superhydrophobic Surface when Collecting Airborne Bacteria. Aerosol Sci. Technol.: Submitted.

ATP-based bioluminescence method

Analysis of the fungal spores collected by liquid samplers is usually performed using microscopy which is not able to analyze low spore concentrations. Some commercial laboratories have developed methods to analyze fungi using PCR techniques; however such techniques have not been applied for sample quantification. As part of this research we successfully developed and applied an ATP-based bioluminescence method to quantify the collected fungal spores. The ATP is a basic energy molecule present in all types of living organisms, and thus it is possible to quantify microbial biomass by measuring the ATP content in a sample using bioluminescence. To quantify spores collected by a sampler being tested one needs calibration curves (luminescence intensity as a function of cell concentration) obtained from the study-specific sampler and target biological particles. The spore concentrations determined by the microscopy and the ATP-based method were not statistically different. In fact, the ATP-based method allowed us to analyze spore concentrations that were too low to reliably detect by microscopy. Thus, the ATP method could serve as a fast and sensitive alternative to traditional microscopy when testing samplers' performance with fungal spores.

The method is described in detail in the following publication:

1. Han, T, Nazarenko, Y, Lioy, PJ and Mainelis, G: [2009]. Collection efficiencies of an electrostatic sampler with superhydrophobic surface for fungal bioaerosols. Environmental Science and Technology: In preparation.

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