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Collection of airborne microorganisms by a new electrostatic precipitator

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Abstract

Bioaerosol exposure assessment and the protection of civil/governmental/military establishments from bioterrorism require the development of low-power bioaerosol collectors that are able not only to efficiently collect airborne microorganisms, but also to preserve their biological integrity. In search for such a method, a new bioaerosol sampler was evaluated. In this device, the airborne microorganisms are imparted electrical charges and are then deposited in an electrical field onto a growth medium (agar). Experiments were conducted with *Pseudomonas fluorescens* vegetative cells, *Bacillus subtilis* var. *niger* (BG) endospores (used to simulate the spores of anthrax-causing *Bacillus anthracis* when testing bioaerosol sensors) and *Penicillium brevicompactum* fungal spores. It was found that 80–90% of initially "charge-neutralized" biological particles were removed from the air, when a small amount of ionization was generated in the electrostatic precipitator's (ESP) inlet and a precipitation voltage of ± 4000 V was applied across the agar plates. Over 70% of viable BG and *P. brevicompactum* spores entering the ESP were enumerated as colony forming units. The bioefficiency of the new sampler was about the same as that of the Biosampler, which was tested in parallel. In experiments with sensitive *P. fluorescens* vegetative cells, the ESP enumerated twice as many cells as the Biosampler. The latter result indicates that the electrostatic collection method may be especially useful for the collection and enumeration of sensitive airborne microorganisms. Experiments investigating the effect of aging time on the amount of electrical charge carried by the airborne microorganisms showed that the level of electrical charge gradually decreases with increasing aging time. However, even after the *P. fluorescens* cells had remained airborne for an hour, they retained enough electrical charge to be collected with efficiency higher than 70%.

Keywords: Electrostatic precipitation; Airborne microorganisms; Collection efficiency; Bioaerosol sampling

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1. Introduction

Exposure to airborne microorganisms in environments such as agricultural, industrial, indoor and healthcare causes millions of episodes of various health complications and disorders in the US each year (Cox & Wathes, 1995). Moreover, there is an increased threat that certain microorganisms, such as anthrax-causing *Bacillus anthracis* spores, may be used as bio-warfare agents. To estimate, control and prevent exposure to airborne biological particles, efficient monitoring and control tools are required.

The concentration of bacteria and fungi in air environments is usually assessed by collecting the microorganisms with impaction or impingement devices and then analyzing the sample. The particle velocity towards the collection medium in these devices is usually tens or hundreds of meters per second, which results in high collection efficiency, but potentially causes loss of viability in sensitive microorganisms (Stewart et al., 1995; Lin et al., 2000). In search for a "more gentle" bioaerosol collection method, we have studied the application of electrostatic precipitation for the collection of viable airborne microorganisms. In electrostatic precipitators (ESP), the particle velocity component perpendicular to the collection medium is two to four orders of magnitude lower than that in bioaerosol impactors and impingers operating at comparable sampling flow rates (Mainelis et al., 1999). Therefore, the electrostatic precipitation technique is potentially less damaging to the microorganisms. In addition, instruments based on this technique can operate at low power input. Low-power bioaerosol collectors are of interest to bioaerosol monitor developers and field practitioners, especially in situations where low-power-consuming monitors are placed in and around buildings and installations to serve as warning devices against bioterrorism.

Our initial attempts to collect airborne microorganisms by electrostatic precipitation showed that hardy microorganisms, such as *Bacillus subtilis* var. *niger* (BG) spores, can be efficiently removed from the air by this technique; sensitive microorganisms, such as vegetative cells of *Pseudomonas fluorescens*, however, suffered a significant loss in viability during the collection process. The loss in viability was attributed to the corona discharge in the ESP's inlet (Mainelis et al., 1999). In our subsequent research we found that the electrical charging of microorganisms is a rather complex process and that several factors need to be taken into account when designing a bioaerosol collector based on electrostatic techniques (Mainelis et al., 2001, 2002a).

Based on our earlier findings on the electro-biological properties of airborne microorganisms, we have designed and built a new electrostatic precipitator for bioaerosol collection (Mainelis, Willeke, Adhikari, Reponen, & Grinshpun, 2002b). In this device, two ionizers charge the incoming biological particles, if they carry insufficient charge for their effective collection. The particles are then subjected to a precipitating electric field and are collected onto two square agar plates placed one after another along the flow axis. When evaluating the performance of this sampler at 4 l/min, we found that initially "charge-neutralized" BG spores and vegetative cells were collected with an overall collection efficiency of approximately 80%, depending on the precipitation voltage. The efficiency obtained for NaCl particles was about 50–60%. When incoming BG spores were charged with positive ions and then collected by a precipitating voltage of ± 1300 V, about 80% of the incoming spores were collected. The overall physical collection efficiency of biological and non-biological particles increased to more than 90% when the particles were electrically charged by the ionizers and the precipitating voltage was increased above ± 4000 V.

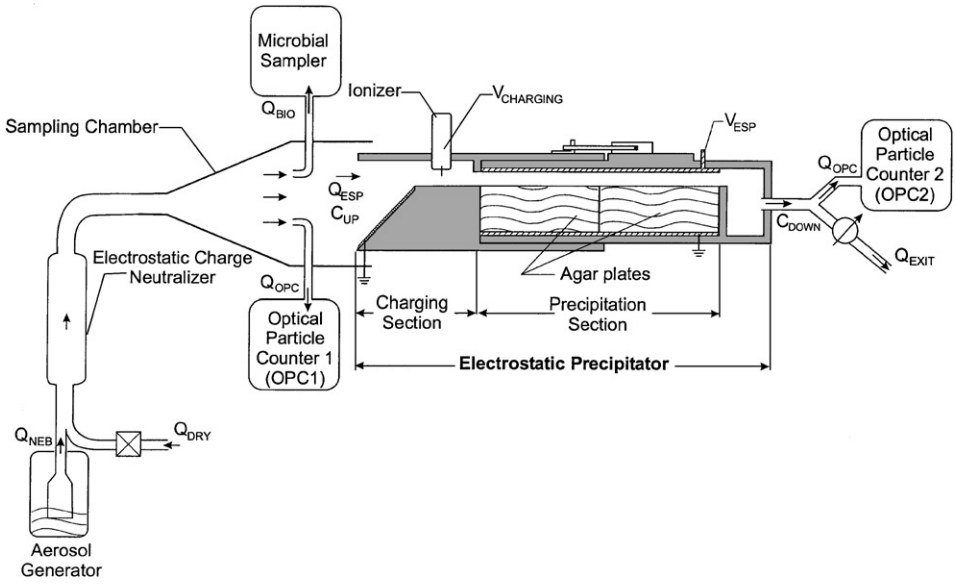

Fig. 1. Experimental setup.

Experiments with BG spores have shown that there are no significant particle losses inside the sampler. The aim of this study (Mainelis et al. 2002b) was to determine the physical sampling efficiency of the new electrostatic sampler, and thus did not address the biological sampling efficiency and microorganism viability.

The present publication focuses on the biological efficiency of the electrostatic sampler, when collecting viable vegetative cells of *P. fluorescens*, bacterial endospores of BG, and fungal spores of *Penicillium brevicompactum*.

2. Materials and methods

2.1. Experimental setup

The experimental setup for this study is shown in Fig. 1. A three-hole Collison nebulizer (BGI Inc., Waltham, Massachusetts) aerosolized the test particles from a liquid suspension at a flow rate of $Q_{NEB} = 4$ l/min. The air entering the nebulizer at a positive pressure of 10 psi was dry and filtered. The resulting aerosol was dried and diluted by a filtered airflow, $Q_{DRY} = 40$ l/min. Since laboratory-generated particles may carry high electrical charges, the electrical charge on aerosolized microorganisms was first reduced (neutralized) to Boltzmann equilibrium charge distribution. The entire airflow of 44 l/min was passed through a 10 mCi Kr-85 charge neutralizer (model 3054, TSI, Inc., Minneapolis, Minnesota). The electrically neutralized particles then entered an open, horizontally oriented sampling chamber from which they were sampled into the ESP.

A detailed description of the ESP has been given elsewhere (Mainelis et al., 2002b); therefore, only its main features are presented here. The device used in our tests (middle of Fig. 1) consists

of a charging section and two plates with the collection medium (e.g., agar) in the precipitation section. In the charging section, the microorganisms enter through the inlet and are then intercepted by positive ions produced by two commercially available ionizers (AS150, Wein Products, Inc., Los Angeles, California). The charging intensity is adjusted by varying the ionizers' voltage, $V_{CHARGING}$. After passing through the inlet/charging section, the microorganisms enter the precipitation section, where they are deposited onto two agar-filled, square Petri dishes (Fisherbrand Square Dishes with Grid, Fisher Scientific, Pittsburgh, Pennsylvania) placed one after another in the flow direction. The intensity of the precipitating field is adjusted by varying the voltage V_{ESP} (0 to ± 5000 V). After the sampling is completed, the charging section is detached from the precipitation section, and the collection plates are removed for subsequent incubation. The particles that do not have a sufficiently high electric charge and are not collected, leave the sampler via the exit. The biological collection efficiency of the ESP was compared to that of the Biosampler (SKC Inc., Eighty Four, Pennsylvania) placed near the inlet of the electrostatic precipitator (shown as "Microbial Sampler" in Fig. 1).

The concentration of particles entering the ESP, C_{UP}, and the concentration of particles leaving the ESP, C_{DOWN}, were monitored by two identical optical particle counters (model 1.108, Grimm Technologies Inc., Douglasville, Georgia), shown as OPC1 and OPC2 in Fig. 1. Each particle counter was operated at a flow rate $Q_{OPC} = 1.2$ l/min and measured the aerosol particle concentrations in 16 size channels, ranging from 0.3 to 20 μm. The airflow through the ESP, Q_{ESP}, was provided by two sources: flow rate Q_{OPC} of OPC2, and the exit airflow, $Q_{EXIT} = Q_{ESP} = Q_{OPC}$. The latter was provided by laboratory-supplied air suction, which was regulated by a control valve. In a field setting, Q_{ESP} is supplied by an external low-power pump or fan.

The entire test system was placed in a Class II, Type B2 biological safety cabinet (Sterilchem-GARD; Baker Company, Sanford, Maine) so that any aerosol particles not collected by the sampler were properly eliminated. All airflow rates were monitored with flow meters (Gilmont Instruments, Inc., Racine, Wisconsin) calibrated with a Buck calibrator (A.P. Buck, Inc., Orlando, Florida).

For a separate set of experiments designed to characterize the effect of bioaerosol aging time on the amount of electrical charge carried by the airborne microorganisms, the entire test system was placed in a 2.6 m^3 walk-in test chamber. In these experiments, the aerosolized *P. fluorescens* vegetative cells did not pass through a charge neutralizer, but directly entered the walk-in chamber.

2.2. Test particles

The new electrostatic precipitator was challenged with three types of biological particles: bacterial vegetative cells of *P. fluorescens*, bacterial endospores of BG, and fungal spores of *Penicillium brevicompactum*.

Vegetative cells of the Gram-negative *P. fluorescens* bacteria are commonly found in ambient air (Nevalainen, 1989; Górny & Dutkiewicz, 1998) and were selected to represent sensitive bacteria (Neidhardt, Ingraham, & Schaechter, 1990). Spores of the Gram-positive bacterium BG are known to be very resistant to many adverse conditions (Sneath, 1986) and thus were selected to represent stress-resistant strains. We have included BG spores in several of our previous studies (Aizenberg, Reponen, Grinshpun, & Willeke, 2000; Lin et al., 2000; Wang, Reponen, Willeke, & Grinshpun, 1999; Wang, Reponen, Grinshpun, Gorny, & Willeke, 2001) because they are used by the Armed Forces in testing new biosensors for their ability to respond to particles of biological threat, such as airborne *B. anthracis* spores. *P. brevicompactum* may cause respiratory allergies (Shen and Han,

1998) and lung diseases, such as farmer's lung (Nakagawa-Yoshida, Ando, Etches, & Dosman, 1997). This species is common in indoor air (Fradkin, Tobin, Tarlo, Tucic-Porretta, & Malloch, 1997; Verhoeff et al., 1990) and has also been reported in the air of some occupational environments (Fischer, Schwalbe, Moller, Ostrowski, & Dott, 1999; Simeray, Mandin, Mercier, & Chaumont, 2001).

Stock cultures of *P. fluorescens* (ATCC 13525) were obtained from the American Type Culture Collection (Rockville, Maryland). *P. fluorescens* is a rod-shaped bacterium with a physical diameter of 0.7–0.8 μm and a length of 2.0–3.0 μm (Palleroni, 1984). The mean aerodynamic diameter of airborne *P. fluorescens* bacteria was found to be 0.8 μm, as reported by Qian, Willeke, Ulevicius, Grinshpun, and Donnelly (1995). The *P. fluorescens* culture was grown in Trypticase Soy Broth (Becton Dickinson Microbiology Systems, Cockeysville, Maryland) at 30°C for 18 h in a Gyrotory Water Bath Shaker (model G76, New Brunswick Scientific Inc., Edison, New Jersey). The cells were harvested from their suspension by centrifugation at $5050 \times g$ for 7 min (Sorval RC-5B, Sorval Co., Newton, Connecticut). The resulting pellets were washed three times with sterile deionized water (5 Stage Milli-Q Plus System, Millipore Corp., Bedford, Massachusetts).

The BG spores were obtained from the US Army Edgewood Laboratories (Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland). BG spores are rod-shaped, approximately 0.7–0.8 μm in width and 1.5–1.8 μm in length (Johnson, Martin, & Resnick, 1994) and have an aerodynamic diameter of ca. 0.8 μm (Qian, Willeke, Grinshpun, & Donnelly, 1997). The BG spores were received in dry form and did not need additional cultivation. Prior to their aerosolization, a small amount of spores was dissolved in deionized, sterilized water. This suspension was then kept at 60°C for 25 min to activate the spores.

The *P. brevicompactum* (CBS 480-84) spores are spherical or slightly ellipsoidal and range from 3 to 4.5 μm in physical diameter (Samson, Hoekstra, Friswald, & Filtenborg, 1995). The mean aerodynamic diameter of aerosolized *P. brevicompactum* spores has been found to be 1.8–2.4 μm (Reponen, Willeke, Ulevicius, Reponen, & Grinshpun, 1996). The *P. brevicompactum* was grown in Petri dishes filled with malt extract agar (Becton Dickinson Microbiology Systems, Sparks, Maryland) at 25°C for 7 days. The spores were separated from the hyphae by adding 0.5 μm glass beads to the Petri dish and slowly shaking the dish for about 10 s (Schmechel, Simpson, & Lewis, 2002). The slow movement of the beads caused the spores to separate from the hyphae, while leaving most of the hyphae intact. The glass beads and the separated spores were then transferred to a vial filled with water. The vial was then shaken, and the beads were let to settle to the bottom of the vial, while the spores remained suspended in the water. This suspension with *P. brevicompactum* spores was then available for aerosolization by a Collison nebulizer. Examination of the suspension under a microscope confirmed that mostly spores and very few hyphae fragments were suspended in the water.

To obtain suspensions of desired microbial density, the initial suspensions of *P. fluorescens* cells, BG endospores and *P. brevicompactum* spores were diluted with deionized, sterilized water. The microorganism concentrations in the air were about 30–50 particles/cm^3 when tests were performed on the overall collection efficiency of the sampler. When the biological performance of the sampler was determined by counting the colony forming units, the concentration of microbial particles in the air was lowered to about 1–5 particles/cm^3 to avoid overloading the growth surface. Trypticase Soy Agar (Becton Dickinson and Co., Cockeysville, Maryland) was used as the collection and growth medium for bacteria, and malt extract agar was used as the collection and growth medium for fungi.

2.3. Experimental procedures

Prior to each experiment, the system was operated without aerosolizing particles until zero particle background was achieved, as measured with the optical particle counters. Next, aerosolization of the biological particles being tested was initiated and the OPC1 readings were checked for aerosol concentration stability in the sampling chamber. The overall physical collection efficiency of the ESP, E_{OVERALL}, was determined from the particle concentrations measured downstream, C_{DOWN}, and upstream, C_{UP}:

$$E_{\mathrm{OVERALL}} = 1 - C_{\mathrm{DOWN}}/C_{\mathrm{UP}}. \quad (1)$$

Our previous research has shown that more than 95% of the airborne *P. fluorescens* vegetative cells and BG spores are registered in three size channels (measuring between 0.5 and 1.0 μm) of the Grimm optical particle size spectrometer (Mainelis et al., 2002a). The overall collection efficiency of the ESP for *P. fluorescens* cells and BG spores has, therefore, been determined using the particle concentrations measured in those channels. Most of the *P. brevicompactum* fungal spores were registered in the three size channels between 1.0 and 3.0 μm. The overall collection efficiency of the ESP sampling *P. brevicompactum* spores has, therefore, been determined using the particle concentrations measured in those channels.

The overall physical collection efficiency of the ESP was determined for all three microbial test particles, at charging voltages $V_{\mathrm{CHARGING}} = 0$ and 1.3 V, and precipitation voltages $V_{\mathrm{ESP}} = -1300$, -4000, $+1300$, and $+4000$ V. The two charging voltages were selected to test the difference between charging ($V_{\mathrm{CHARGING}} = 1.3$ V) and no charging ($V_{\mathrm{CHARGING}} = 0$ V) of the sampled microorganisms. At least three repeats were performed for each experiment. The charging voltage of 1.3 V resulted in an ionization current per ionizer of 115 mA. The selection of the precipitation and charging voltages was based on our previous research (Mainelis et al., 2002b), which showed that precipitation voltages of ± 4000 V ensured an overall physical collection efficiency of about 70% when no charging was applied; precipitation voltages of ± 1300 V ensured an overall physical collection efficiency of about 90% when electrical charging at $V_{\mathrm{CHARGING}} = 1.3$ V was applied to the incoming airborne microorganisms.

The experiments were performed at sampling flow rate $Q_{\mathrm{ESP}} = 4$ l/min which was shown in earlier tests to result in an overall physical collection efficiency of 70–80% even when no external charging was applied to the incoming microorganisms (Mainelis et al., 2002b). Since the ESP is essentially an open channel with a small pressure drop, this airflow rate can be supplied in the field by a portable, low-power pump. Currently, such pumps are capable of maintaining an airflow of 4 l/min for up to 8–10 h. The sampling flow rate of 4 l/min is often used in personal aerosol sampling devices, e.g. the Button Inhalable Aerosol Sampler (Aizenberg et al., 2000). The performance of the ESP can thus be compared to that of personal aerosol samplers operating at the same sampling flow rate. At this sampling flow rate, the average air flow velocity through the ESP is 14.3 cm/s.

Since E_{OVERALL} is calculated from the entering and exiting aerosol concentrations in the ESP, this parameter does not differentiate between the collected particles that are viable and those that are or have become non-viable. However, when sampling airborne microorganisms, only the viable particles deposited on the growth medium will form colonies, which, in turn, will be used to determine the concentration of viable microorganisms sampled. In a strict sense, the viable number, when determined by cultivation, is more a measure of the microorganism's ability to grow on the chosen

medium than of the actual viable number in the sample (Henningson, Lundquist, Larsson, Sandstrom, & Forsman, 1997). However, for consistency sake, we will be using the term "viable" throughout the text. Therefore, the collection efficiency of any sampler designed for viable microorganisms is defined as the fraction of viable airborne microorganisms upstream of the sampler that has been collected and has formed colony forming units. In our experiments, the efficiency of collecting and maintaining viable microorganisms in the ESP, i.e., its bioefficiency, E_{CFU}, was determined by counting the number of colony forming units on the agar and comparing this number with the number of viable particles entering the sampler:

$$E_{CFU} = N_{CFU}/(C_{UP} t Q_{ESP} F_{INITIAL}), \quad (2)$$

where N_{CFU} is the number of colonies formed, C_{UP} is the number of particles entering the sampler as measured by the Grimm optical particle size spectrometer, t is the sampling time (2 min for the ESP), and $F_{INITIAL}$ represents the fraction of viable microorganisms in the initial nebulizer suspension. E_{CFU} was determined for the same experimental parameters as for the overall physical collection efficiency of the ESP: all three microbial test particles; charging voltages $V_{CHARGING} = 0$ and 1.3 V; precipitation voltages $V_{ESP} = -1300$, -4000, $+1300$, and $+4000$ V; sampling flow rate $Q_{ESP} = 4$ l/min.

The bioefficiency of the ESP was compared to that of a microbial sampler (Biosampler, SKC Inc., Eighty Four, Pennsylvania), which was run in parallel to the ESP and collected airborne microorganisms into sterile deionized water. The collection efficiency of the Biosampler was determined using Eq. (2) with Q_{ESP} replaced by $Q_{BIO} = 12$ l/min and sampling time $t = 5$ min.

After completing the sampling, the agar plates from the ESP were removed and incubated; aliquots from the Biosampler sampling liquid were diluted, cultivated on agar in triplicate and incubated. The plates with *P. fluorescens* vegetative cells, BG endospores, and *P. brevicompactum* spores were incubated for 48 h at 30°C. After incubation, the colonies formed were counted, from which N_{CFU} was determined for each sample.

$F_{INITIAL}$ was determined prior to aerosolization as the ratio of the number of viable microorganisms in the suspension, $N_{CULTURABLE}$, to the total number of microorganisms in the suspension, N_{TOT}:

$$F_{INITIAL} = N_{VIABLE}/N_{TOT}. \quad (3)$$

N_{VIABLE} was determined by taking aliquots from the initial suspensions, and then diluting and cultivating them in triplicate on agar media. Only the counts from dilutions that resulted in 30 to 300 colonies per plate were used to obtain the average count of viable microorganisms.

The total number of microorganisms in each suspension was determined by staining the microorganisms with acridine orange (Sigma Chemical Co., St Louis, Missouri) and subsequently counting them under an epifluorescence microscope (model Laborlux S; E. Leitz, Inc., available from Nuhsbaum Inc., McHenry, Illinois).

When measuring the effect of microorganism aging time on the amount of electrical charge carried by the airborne microbial particles, *P. fluorescens* vegetative cells were aerosolized into the walk-in test chamber without passing through the charge neutralizer. The cells were then collected by the ESP every 15 min for 90 min and the overall physical collection efficiency of the ESP, $E_{OVERALL}$, was determined at four collection voltages, $V_{ESP} = -75$, -200, -500, -3000 V. No additional charging was used in these experiments ($V_{CHARGING} = 0$ V).

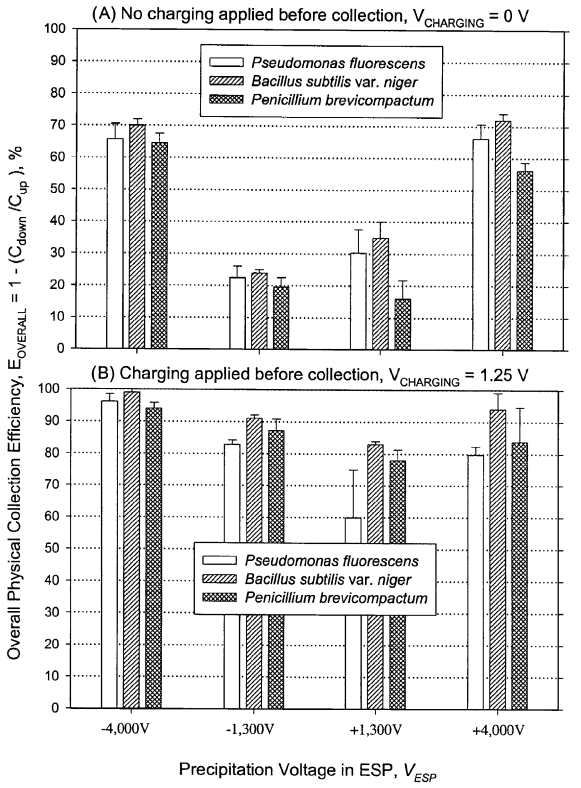

Fig. 2. Overall physical collection efficiency of the new ESP, as determined by comparing the particle concentrations upstream and downstream of the sampler. After aerosolization the microorganisms were passed through a charge neutralizer prior to being sampled. Measurements were performed with (A) and without (B) electrical charging applied. ESP sampling flow rate, Q_{ESP}, =4 l/min. The error bars represent the standard deviation for three repeats.

2.4. Data analysis

The data analysis was performed using analysis of variance (ANOVA) available as an add-in to Microsoft Excel 2000. P values of < 0.05 were considered significant.

3. Results and discussion

Fig. 2 shows the results from the first set of experiments, in which the overall physical collection efficiency of the ESP, $E_{OVERALL}$, was determined for sampling biological particles at different precipitation voltages. Fig. 2A presents the data obtained when no charging was applied before the collection, and Fig. 2B presents the data obtained with additional charging of $V_{CHARGING} = 1.3$ V applied. The error bars represent the standard deviation for at least three repeats. These tests were conducted with the microorganisms passed through an electrical charge neutralizer after aerosolization. When no charging was applied ($V_{CHARGING} = 0$ V) and the ESP operated at a precipitation

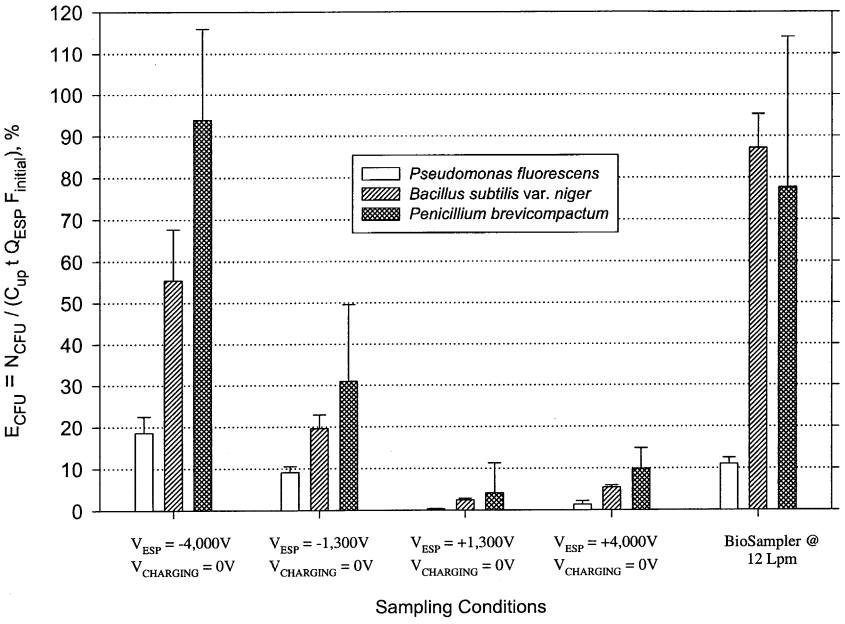

Fig. 3. The biological collection efficiency of the new ESP as determined by the CFU count when sampling three different microorganisms at different precipitation voltages. After aerosolization the microorganisms were passed through a charge neutralizer prior to being sampled. $Q_{ESP} = 4$ l/min; no charging applied in ESP. The measured efficiency is compared to that of the Biosampler operated at 12 l/min. The error bars represent the standard deviation for three repeats.

voltage of ± 1300 V, about 20–30% of the airborne microorganisms entering the sampler were removed from the air inside the ESP. When the precipitation voltage was increased to ± 4000 V, about 60–70% percent of the microorganisms were collected inside the ESP (Fig. 2A). At the lower precipitation voltage of ± 1300 V, the overall physical collection efficiency of the ESP increased from 30% to at least 60% when the incoming microorganisms were additionally charged ($V_{CHARGING} = 1.3$ V). When the precipitation voltage was increased to ± 4000 V, while maintaining the same charging conditions, more than 80% of microorganisms were removed from the air for all three species (Fig. 2B). These data show that the new electrostatic sampler can effectively collect even the "charge-neutralized" airborne microorganisms, if the collection voltage is high enough (Fig. 2A), e.g., ± 4000 V in our tests. When the incoming particles are additionally charged, they can be removed from the air efficiently even at lower precipitation voltages.

In the next set of experiments we determined the efficiency of collecting and enumerating viable microorganisms in the ESP for three test microorganisms without any additional charging applied (Fig. 3). As in the tests described earlier, the microorganisms passed through the electrical charge neutralizer after aerosolization, prior to being sampled. The lowest bioefficiencies, i.e., the lowest ratios of the number of colonies formed to the number of viable microorganisms entering the sampler, were achieved at positive precipitation voltages of 1300 and 4000 V. For these two voltages, the lowest $E_{CFU}(< 3\%)$ was found for *P. fluorescens* bacteria, and the highest (5–10%) for *P. brevicompactum* fungal spores. A significantly higher E_{CFU} was measured when the microorganisms were collected at $V_{ESP} = -1300$ V. In this case, cells of *P. fluorescens*, endospores of BG, and

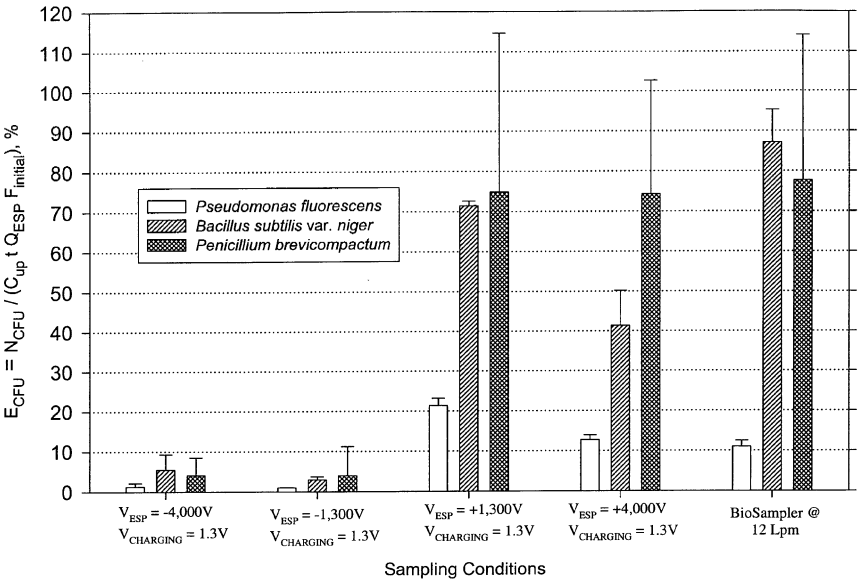

Fig. 4. The biological collection efficiency of the new ESP as determined by the CFU count when sampling three different microorganisms at different precipitation voltages. After aerosolization the microorganisms were passed through a charge neutralizer prior to being sampled. $Q_{ESP} = 4$ l/min, $V_{CHARGING} = 1.3$ V. The measured efficiency is compared to that of the Biosampler operated at 12 l/min. The error bars represent the standard deviation for three repeats.

spores of *P. brevicompactum* were collected and enumerated with efficiencies of 10%, 20%, and 30%, respectively. When the precipitation voltage was increased to $V_{ESP} = -4000$ V, the cells of *P. fluorescens*, endospores of BG, and spores of *P. brevicompactum* were collected and enumerated with bioefficiencies of about 20%, 55%, and 90%, respectively. This increase in the E_{CFU} was statistically significant ($p < 0.05$) for all three microbial species.

When comparing the biological efficiency of collecting viable microorganisms with the ESP (without additional charging applied) with that of the Biosampler (Fig. 3), the following was observed. At ESP precipitation voltages of -1300, $+1300$, and $+40000$ V, the biological collection efficiencies of the ESP are much lower than those of the Biosampler. These differences are statistically significant ($p < 0.05$) for all the tested species and precipitation voltages, with the exception of *P. fluorescens* collected at $V_{ESP} = -1300$ V. On the other hand, for the higher precipitation voltage of -4000 V, the biological collection efficiency of microorganisms in the ESP is higher than that of the Biosampler when collecting sensitive *P. fluorescens* cells ($p < 0.05$); somewhat lower when collecting BG spores ($p < 0.05$); and not statistically different when collecting *P. brevicompactum* spores ($p > 0.05$). It appears that the ESP bioefficiency when collecting microorganisms without additional charging was closest to that of the Biosampler at $V_{ESP} = -4000$ V.

Fig. 4 shows the biological efficiency of viable microorganisms in the ESP at $V_{CHARGING} = 1.3$ V. As in the previous experiments, the microorganisms passed through the electrical charge neutralizer after aerosolization, prior to being sampled. The lowest bioefficiencies were achieved when the microorganisms were positively charged and collected at negative precipitation voltages of -1300 and -4000 V. For both voltages and for all three microorganisms, E_{CFU} was about 5% or less.

When the precipitation voltage was switched from negative to positive ($V_{ESP} = +1300$ V), a large and statistically significant ($p < 0.05$) increase in E_{CFU} was observed. In this case, the cells of *P. fluorescens*, endospores of BG, and spores of *P. brevicompactum* were collected and enumerated with efficiencies of approximately 20%, 70%, and 75%, respectively. When the positive precipitation voltage was further increased to $V_{ESP} = +4000$ V, the E_{CFU} for the *P. fluorescens* cells and BG spores decreased to 12% and 42%, respectively. Both decreases were statistically significant ($p < 0.05$). The E_{CFU} was essentially unchanged for the *P. brevicompactum* spores.

Fig. 4 also shows the biological collection efficiencies of the Biosampler. For ESP precipitation voltages of -1300 and -4000 V, the ESP collects fewer viable microorganisms than the Biosampler. These differences were found to be statistically significant ($p < 0.05$) for all species and for both precipitation voltages.

On the other hand, when the microorganisms were positively charged and collected at a positive precipitation voltage of $+1300$ V, the ESP collected and enumerated as many (or, in the case of sensitive *P. fluorescens* cells, even more) microorganisms as the Biosampler. This result was statistically significant ($p < 0.05$). For this precipitation voltage, the E_{CFU} of the ESP is very close to the overall physical collection efficiency of the device when collecting BG and *P. brevicompactum* spores (Fig. 2), i.e., most of these microorganisms collected onto the agar surfaces in the ESP formed colonies. For sensitive *P. fluorescens* cells, which may suffer significant stress during their aerosolization and air transport, only about a third of the collected cells form colonies. However, the ESP collected and enumerated about twice as many of these sensitive cells ($p < 0.05$) as the Biosampler.

When comparing the biological collection efficiencies of viable microorganisms in the ESP, presented in Figs. 3 and 4, conclusions can be drawn about the processes taking place inside the electrostatic sampler. Our earlier studies (Mainelis et al., 2001) have shown that aerosolized *P. fluorescens* cells and BG spores carry a net negative charge, i.e., most of the microorganisms are negatively charged. Even after passage through an electric charge neutralizer the microorganism populations appear to maintain a net negative charge, although the magnitude of the charges is greatly reduced. When these microorganisms enter the ESP, while no external charging is applied (Fig. 3), they pass through the charging section retaining their net negative charge distribution and then enter the precipitation section. If a negative precipitation voltage of -1300 V is applied to the upper electrode in the ESP (Fig. 1), many or most of the negatively charged microorganisms are repelled towards the collection medium, where they can form colonies (Fig. 3). Once a higher negative precipitation voltage of -4000 V is applied, many more of the negatively charged microorganisms are repelled towards the collection medium and form colonies. Thus, the biological collection efficiency increases with increasing negative precipitation voltage (Fig. 2, and two bar groups on left in Fig. 3). Among the microorganisms entering the ESP, some carry a positive electrical charge. Thus, when a positive precipitation voltage is applied to the upper electrode, those few microorganisms are repelled towards the collection medium, where they can grow and form colonies. Due to the low number of positively charged microorganisms, however, the bioefficiency is very low when sampling at a positive precipitation voltage.

The efficiencies for collecting and enumerating viable microorganisms at positive precipitation voltages (Fig. 3) are much lower than the overall collection efficiencies (Fig. 2A) measured under the same sampling conditions. We interpret this difference as follows: the negatively charged microorganisms entering the sampling section are attracted to the upper plate which, in this case, is kept

at a positive electrical potential. These negatively charged particles, which constitute the majority of the particles in the sampler, are not registered downstream of the sampler. Thus, a high overall physical collection efficiency is observed. Since only microorganisms collected on the agar plates (lower electrode) can form colonies, the microorganisms collected on the upper electrode do not contribute towards the efficiency of viable microorganism collection. The same applies to negatively charged microorganisms subjected to a positive precipitation field: these microorganisms are attracted towards the upper electrode and contribute to the overall physical collection efficiency of the ESP, but not to the device's bioefficiency.

Fig. 3 shows that the number of negatively charged particles entering the ESP was much higher than the number of positively charged particles for all three tested microorganism species. However, the ratio of negatively versus positively charged particles may be quite different in a field situation. Therefore, when the ESP is used to collect airborne microorganisms without additional charging applied, the data obtained at identical positive and negative voltages should be added together. As seen in Fig. 3, summation of the bioefficiency data obtained at identical negative and positive precipitation voltages results in an increase of the ESP bioefficiency.

When the microorganisms are imparted positive electrical charges through the ionizers in the inlet section, their electrical charge distribution changes, i.e., most of the microorganisms become positively charged (Fig. 4). When a positive precipitation voltage of 1300 V is applied to the top electrode, the majority of the particles will be repelled towards the collection medium, placed on and electrically connected to the bottom electrode. There the microorganisms can form colonies, and will contribute towards the bioefficiency of the ESP (Fig. 4, middle bar group). Those few microorganisms that remain negatively charged can be collected by a negative precipitation voltage (Fig. 4, two left bar groups). The sum of data obtained at negative and positive precipitation voltages represents the total number of viable microorganisms that can be collected by electrostatic forces.

When the ESP was operated at $V_{ESP} = +1300$ V and $V_{CHARGING} = 1.3$ V, its bioefficiency for viable microorganism collection was within 5–10% of the Biosampler's bioefficiency (Fig. 4). Moreover, under these conditions, the number of sensitive *P. fluorescens* bacteria enumerated by the ESP was higher than the number enumerated by the Biosampler. The Biosampler is known to induce minimal stress to the microorganisms sampled (Lin et al., 2000). Apparently, as hypothesized in our earlier research (Mainelis et al., 1999), the electrostatic precipitation method induces even less stress to sensitive microorganisms, i.e., it is a "more gentle" collection method.

Fig. 4 also shows that the bioefficiency of the ESP decreased for both bacterial species, as the precipitation voltage increased from +1300 to +4000 V; on the other hand, the overall physical collection efficiency at these conditions increased. Apparently, some of these bacterial particles acquired very high positive electrical charges and became highly mobile in the applied electrical field. The higher electrostatic precipitation field caused many of these microorganisms to deposit on the grounded surface before they could reach the growth medium. Observation of a high density of microorganisms deposited near the leading edge of the agar plate closest to the inlet supports this argument. The particles deposited ahead of the agar plate contributed to the overall physical collection efficiency, but not to the bioefficiency. The larger *P. brevicompactum* fungal spores, on the other hand, had higher inertia and even an increased electrical field did not cause their "premature" collection on the grounded surface. Therefore, for field applications, the design of the ESP may have to be modified to prevent the "premature" collection of microorganisms with high electrical mobility.

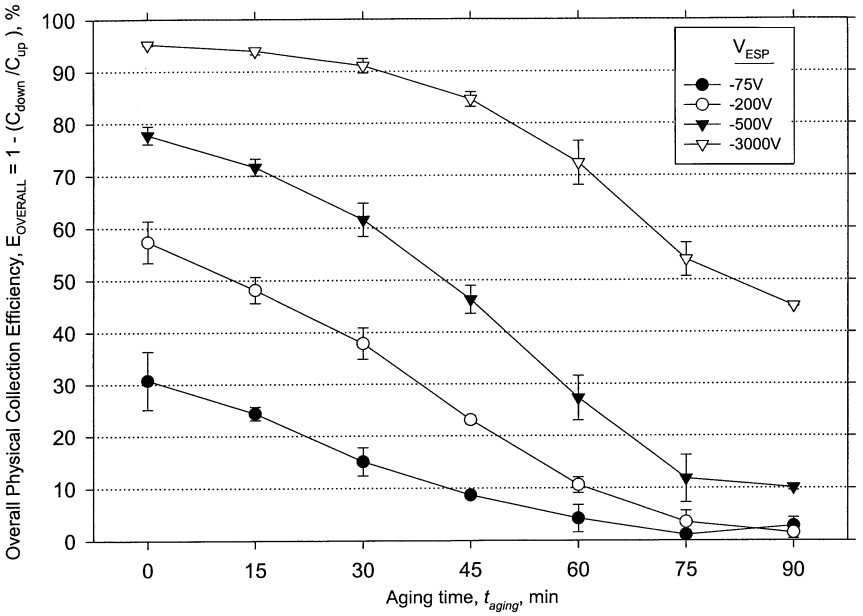

Fig. 5. Overall physical collection efficiency of the ESP when collecting *P. fluorescens* vegetative cells right after their dispersion and after aging them in a chamber for time t_{aging}. ESP sampling conditions: $Q_{ESP} = 4$ l/min, $V_{CHARGING} = 0$ V. The microorganisms were collected on TSA agar. The error bars represent the standard deviation for three repeats.

In further applications of the ESP, the ionizers may have to be moved closer to the first agar plate or to the front edge of agar plates placed opposite to each other on each electrode.

Our earlier research has shown that airborne microorganisms may carry thousands of elementary electrical charge units immediately after their aerosolization (Mainelis et al., 2001). However, until now it has not been known how long this electrical charge is maintained, if the microorganisms remain suspended in the air for some time. As Figs. 2 and 3 show, the tested airborne microorganisms retained sufficient electrical charge for efficient collection by the ESP even after passage through a radioactive charge neutralizer. Thus, one may ask whether aged bioaerosols will retain a high enough electrical charge, so that they can be collected by the electrostatic technique without additional charging. This would greatly simplify the collection device and would eliminate potential microorganism stress due to the charging process.

Thus, in our next experiment we determined how aging time affects the amount of electrical charge on the airborne microorganisms. We measured the overall physical collection efficiency of the ESP collecting *P. fluorescens* vegetative cells immediately after their dispersion and after aging them up to 90 min. Since the overall physical collection efficiency of the ESP depends on the amount of electrical charge carried by the airborne particles, changes in the efficiency with time indicates changes in the amount of electrical charge carried by the microorganisms. The results of this experiment are presented in Fig. 5. Immediately after their aerosolization the *P. fluorescens* cells had a high electrical charge so that more than 50% of them were collected at a low precipitation voltage of −200 V; more than 95% of the cells were removed from the air when the precipitation voltage was increased to −3000 V. After the bioaerosol had been exposed to the ever present atmospheric

radiation, i.e. "aged", for 30 min, the magnitude of the electrical charge carried by the microorganisms was somewhat reduced. Therefore, comparatively smaller fractions of the microorganisms were collected at any of the four precipitation voltages used in the tests. However, even after 30 min, application of $V_{ESP} = -500$ V still resulted in the removal of more than 60% of the microorganisms in the air; application of $V_{ESP} = -3000$ V resulted in a particle removal efficiency higher than 90%. After the bioaerosol had aged for 60 min, the electrical charge on the microorganisms had decreased further and only 28% of the microorganisms were removed from the air at $V_{ESP} = -500$ V. However, application of the higher precipitation voltage $V_{ESP} = -3000$ V still resulted in an overall physical collection efficiency higher than 70%. This efficiency decreased to about 45% after the *P. fluorescens* cells had aged for 90 min. The above results show that the amount of electrical charge carried by the airborne microorganisms decreases with increasing bioaerosol aging time. Nevertheless, even after 90 min the microbial particles still carry enough electrical charge so that they can be effectively removed from the air, if a sufficiently high precipitation voltage is used.

4. Conclusions

This research showed that the newly designed electrostatic precipitator (Mainelis et al., 2002b) is capable of efficiently collecting and enumerating airborne microorganisms. Up to 70% of "charge-neutralized" biological particles were removed from the air, when a precipitation voltage of ± 4000 V was applied. The overall collection efficiency of the ESP was found to increase to 80–90% (depending on the magnitude of the precipitation voltage) when a small amount of ionization was applied in the ESP inlet. Experiments with *P. fluorescens* vegetative cells, *B. subtilis* var. *niger* (BG) endospores and *P. brevicompactum* fungal spores showed that these microorganisms carry a net negative electric charge even after passing through a 10 mCi Kr-85 charge neutralizer. The amount of this residual charge was high enough for the microorganisms to be efficiently collected and enumerated in a negative precipitation field when no additional charging was applied. For microorganisms of unknown origin, electrical fields of both polarities, negative and positive, may have to be used for their collection. In future applications of the electrostatic collection method, the present ESP prototype may have to be modified to allow for simultaneous enumeration of negatively and positively charged microorganisms.

When BG endospores and *P. brevicompactum* fungal spores were sampled by the ESP with a small amount of positive ionization in the inlet and collection in a positive precipitation field, they were enumerated with an efficiency that was within 5–10% of that obtained by use of the Biosampler. The number of sensitive *P. fluorescens* cells was about twice as high when counted in the ESP compared with the Biosampler. This result shows that the electrostatic technique not only collects airborne microorganisms efficiently, but also better preserves the viability of sensitive microorganisms.

Experiments investigating the effect of aging time on the amount of electrical charge carried by the airborne microorganisms showed that the level of electrical charge gradually decreases with increasing aging time. However, even after the *P. fluorescens* cells had remained airborne for an hour, they retained enough electrical charge to be collected with efficiency higher than 70%. Thus, when the electrostatic collection technique is used to collect airborne microorganisms in various environments, it is quite possible that the microorganisms may be effectively collected and enumerated in many

of these environments without applying any additional charging. This approach would simplify the design of electrostatic samplers and such devices could be used at minimum electrical power. This is of interest when low power consuming monitors are placed in and around buildings and installations, e.g., as warning devices against bioterrorism.

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