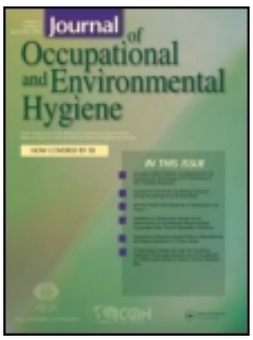

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Assessment of Electrical Charge on Airborne Microorganisms by a New Bioaerosol Sampling Method

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Bioaerosol sampling is necessary to monitor and control human exposure to harmful airborne microorganisms. An important parameter affecting the collection of airborne microorganisms is the electrical charge on the microorganisms. Using a new design of an electrostatic precipitator (ESP) for bioaerosol sampling, the polarity and relative strength of the electrical charges on airborne microorganisms were determined in several laboratory and field environments by measuring the overall physical collection efficiency and the biological collection efficiency at specific precipitation voltages and polarities. First, bacteria, fungal spores, and dust dispersed from soiled carpets were sampled in a walk-in test chamber. Second, a simulant of anthrax-causing Bacillus anthracis *spores was dispersed and sampled in the same chamber. Third, bacteria were sampled in a small office while four adults were engaged in lively discussions. Fourth, bacteria and fungal spores released from hay and horse manure were sampled in a horse barn during cleanup operations. Fifth, bacteria in metalworking fluid droplets were sampled in a metalworking simulator. It was found that the new ESP differentiates between positively and negatively charged microorganisms, and that in most of the tested environments the airborne microorganisms had a net negative charge. This adds a signature to the sampled microorganisms that may assist in their identification or differentiation, for example, in an anti-bioterrorism network.*

Keywords airborne microorganisms, bioaerosol sampling, collection efficiency, electrostatic precipitation, metalworking fluid

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INTRODUCTION

Although microorganisms exist everywhere in our daily life,[1] some of them are particularly harmful to human health. Exposure to pathogenic, allergenic, and toxic airborne microorganisms may cause a wide range of respiratory disorders and health effects,[2,3] such as respiratory irritation and infection, asthma and allergy, extrinsic allergic alveolitis, organic dust syndrome, and chronic bronchitis.[4] To control and prevent such health impairments, precise and fast methods are needed for the speciation of airborne microorganisms and the determination of their concentrations. Improved bioaerosol sampling techniques are particularly urgently needed for the establishment of warning networks against bioterrorism.[5]

To find improved measurement methods, additional features need to be discovered that are unique to specific microorganisms and can be used as signatures in their detection and concentration monitoring. This study was undertaken to find a technique for measuring the magnitude and polarity of electrical charges on airborne microorganisms. The technique used is also a promising new method for bioaerosol sampling.

The most common samplers used to collect airborne microorganisms are bioaerosol impingers and impactors.[6,7] In these devices the particle velocity perpendicular to the collection medium is tens or hundreds of meters per second. This high velocity generally results in high physical collection efficiency but may damage the viability of sensitive microorganisms.[8,9] Particle removal by exposing the aerosol stream to an electrostatic field is potentially more gentle for airborne microorganisms because the particle removal velocity toward the collection medium perpendicular to the airflow is about two to four orders of magnitude lower than that in bioaerosol impactors and impingers at comparable sampling rates.[10] In this study, electrical forces were used to collect airborne microorganisms and to determine the amount and polarity of electrical charges attached to or embedded in them.

From prior studies on airborne microorganisms it can be concluded that many airborne microorganisms naturally carry electrical charges in their outer shell; for example, some of the airborne organic particles in Antarctica have been found

to be positively charged, which affects their deposition to the ground depending on the strength of the electric field that is naturally present in the air.[11] Our previous laboratory experiments have shown that bacterial cells and spores carry higher levels of electrical charge than inert particles.[12] These tests also showed that the laboratory-generated microorganisms had a wide distribution in the number of positive or negative electric charges carried by them, but each ensemble of test microorganisms had a net negative charge.[12] The present laboratory and field study gives information on the polarity and magnitude of electrical charges on airborne microorganisms through examination of their physical collection efficiency and microbial culturability in our newly developed electrostatic precipitator.

The viability of airborne particles may be affected by the corona discharge in a conventional electrostatic precipitator (ESP) used for collecting nonbiological particles. For instance, through experiments conducted with a modified conventional ESP, we found that sensitive bacteria such as *Pseudomonas fluorescens* lost their viability, while hardy bacteria such as *Bacillus subtilis* var. *niger* (BG) endospores (anthrax simulant) were not affected by the ion current in the corona discharge.[10,13] Based on our findings on the electro-biological properties of airborne microorganisms, we have developed a new, low-power ESP for bioaerosol collection that can be operated with or without an ionizer in the entrance section.[14] This study presents data on the electric charge properties of collected microorganisms using this ESP.

MATERIALS AND METHODS

Experimental Set-Up and Materials

In this study, the electrical charges on airborne microorganisms were assessed in field environments and in controlled environments simulating field conditions: bacteria, fungal spores, and dust dispersed from heavily used carpets were sampled in a walk-in test chamber; bacteria simulating *Bacillus anthracis* spores were dispersed and sampled in the same chamber; bacteria dispersed by people were sampled in a small office with four adults engaged in lively discussions; bacteria and fungal spores in hay and horse manure were sampled in a horse barn during cleanup operations; and bacteria in droplets dispersed from metalworking fluids were sampled in a simulation facility for metalworking operations.

The experiments on the dispersion of bacteria, fungal spores, and dust from used carpets were performed in a 2.6 m^3 walk-in test chamber with glass windows built into the walls. Figure 1 shows a schematic representation of the walk-in chamber and the experimental set-up in the chamber. All operations were performed from outside the chamber by inserting hands into gloves mounted and sealed into the chamber walls. A strip of heavily used carpet was placed onto the bottom of the walk-in chamber and a vacuum cleaner nozzle with beater bar was placed onto it. The nozzle was moved back and forth by a long rod handled from outside the chamber. The air was moved through the nozzle in the reverse direction at about 1,600 L/min by connecting the nozzle to the outlet of a vacuum cleaner

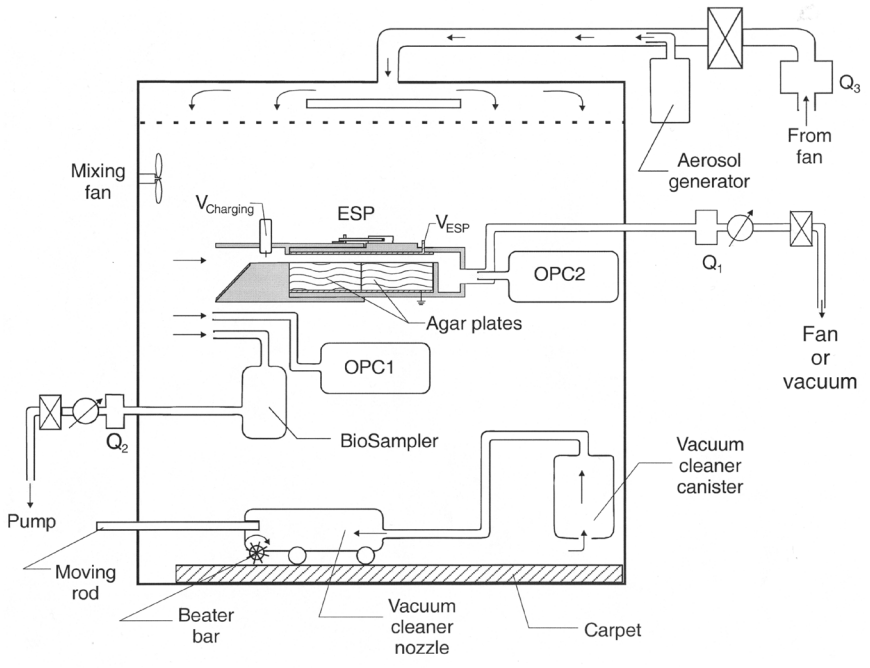

FIGURE 1. Schematic representation of walk-in chamber and experimental set-up for sampling bacteria, fungal spores and dust dispersed from used carpets. The chamber was also used for dispersing and sampling *Bacillus subtilis* var *niger* (BG) spores (anthrax simulant)

canister; thus, the carpet-embedded microorganisms and dust particles were dispersed into the chamber by beater bar action and airflow or, if the beater bar was turned off, by airflow alone.

The airborne microorganisms and dust particles in the chamber were sampled by a new electrostatic precipitator, a BioSampler (model 225-9595, developed in our laboratory; available through SKC Inc., Eighty Four, Pa.) and two optical particle counters (model 1.108, Grimm Technologies Inc., Douglasville, Ga.). The BioSampler has well-established performance characteristics[9] and was used as a reference sampler for the colony-forming unit (CFU) count from the ESP.

We would have preferred the Andersen sampler as the reference sampler; however, tests with the Andersen sampler resulted in overloaded plates, even when the CFU count in the ESP was low. The BioSampler was chosen because it could be used for the same sampling times as the ESP, thus eliminating any differences in nonconstancy of the aerosol concentration. Only when used with collison-nebulized spores was the sampling time different for the two devices. In this case, however, the Grimm optical particle counter (OPC) showed that the aerosol concentration was constant within 3% during the sampling time. Tests with a Burkard sampler on the laboratory-generated microorganisms showed that the airborne microorganisms were essentially monodisperse. Less than 5% of the collected microorganisms were attached to each other as doublets or triplets.

The Grimm OPCs allowed us to measure the aerosol concentrations in the size range of the airborne microorganisms. Both Grimm counters were factory-calibrated. Independent tests in our laboratory confirmed that both devices measured the same aerosol concentration within 5%. The BioSampler and one Grimm optical particle counter (OPC1) were positioned directly under the inlet of the electrostatic precipitator. Measurements with the Grimm counter showed that all three samplers faced essentially the same aerosol concentration (within 5%) at their inlets.

The second Grimm counter (OPC2) sampled from the outlet of the ESP so that the number of particles collected in the ESP could be determined from the difference in count between OPC1 and OPC2. OPC2 sampled from the ESP exit flow at approximately isokinetic conditions.[15] The two identical particle counters recorded the aerosol concentrations in sixteen particle size channels, ranging from 0.3 μm and 20 μm, and were operated at a flow rate of 1.2 L/min each. Because the size range of interest for fungi and bacteria is below about 5 μm, only the first 10 channels from 0.3 μm to 5 μm were used for the determination of the overall physical collection efficiency. All of the flow rates, Q_1, Q_2, and Q_3 in Figure 1, were monitored by flow meters (Gilmont Instruments, Inc., Racine, Wis.), calibrated with a Buck calibrator (A.P. Buck, Inc., Orlando, Fla.).

The new electrostatic precipitator used in our experiment consists of an inlet section containing two ionizers (model AS 150, Wein Products, Inc., Los Angeles, Calif.), and a precipitation section housing two square petri dishes (Fisher-brand square dishes with grid, Fisher Scientific, Pittsburgh, Pa.) filled with the collection medium (e.g., agar). The petri dishes are made of plastic. An aluminum foil is wrapped around each petri dish from the top edge of the agar to the external bottom of the dish, which sits on a grounded metal plate. Thus, the electrically conducting agar surface is connected to ground potential. The two ionizers allow us to add electrical charges to the sampled microorganisms, if they carry insufficient charges for efficient collection in the ESP's precipitation section. The principle and detailed design of this new device are given elsewhere.[14]

The ESP was operated at a flow rate of 4 L/min. Because OPC2 in the exhaust flow of the ESP sampled at a flow rate of 1.2 L/min, a small fan or a vacuum source exhausted an additional flow Q_1 of 2.8 L/min (see Figure 1). The ionization current is adjusted by changing the ionizers' voltage, $V_{Charging}$. After passage through the charging section, the microorganisms and dust particles enter the precipitation section where they are exposed to an electrostatic field that is established through voltage V_{ESP} on the upper electrode, while the lower electrode is grounded. The voltage is set to a potential that is sufficient for collecting the microorganisms onto the two agar-filled square petri dishes, placed one after the other. For our evaluation tests, we chose three typical voltage levels that differed from each other by a factor of 3: $\pm$300 V, $\pm$1000 V, and $\pm$3000 V. The top of the agar surface is about 5 mm below the upper electrode.

The same walk-in chamber was also used to disperse and sample bacteria simulating *B. anthracis* spores. A three-hole Collison nebulizer (BGI Inc., Waltham, Mass.) was used as the aerosol generator for endospores of *B. subtilis* var. *niger* (BG), which were dispersed from a liquid suspension of deionized and sterilized water at a flow rate of 6 L/min (see Figure 1). Dried and filtered air was supplied to the Collison nebulizer at 12 psi (83×10^3 Pa). Filtered, dry air of 40 L/min was mixed with the aerosol flow so that the liquid in the droplets evaporated, and only the BG spores and much smaller droplet residues entered the test chamber. The BG spores (cultured by and obtained from the U.S. Army Edgewood Research, Development and Engineering Center; Aberdeen Proving Ground, Md.) were essentially monodispersed and not charge neutralized. Passage of the aerosol through perforated metal sheets at the top and bottom inside the chamber ensured uniformity of the aerosol distribution throughout the chamber. A fan on the inner chamber wall helped circulate and mix the air inside the chamber. The air sampling was performed in the same manner as in the carpet experiment.

In one of the field studies, the ESP and a BioSampler were placed on a small conference table in an office in the presence of four adults. In another field study, the ESP and BioSampler were placed about 1 m above the floor in a horse barn, while hay and manure were being moved during a routine cleanup operation of two stalls.

Finally, we sampled bacteria in air environment near a metalworking operation. In an industrial metalworking operation, the metalworking fluid is usually recycled through sumps, which allows the buildup of bacterial contamination.[3] To

perform a controlled experiment, we built a small metalworking simulation facility and placed it inside a biological safety cabinet (Sterilchem-Gard Class II, Type B2, Baker Co., Sanford, Maine). A typical metalworking fluid was used, consisting of water and 5% semisynthetic concentrate (43BKH-2 without biocide, Milacron Inc., Cincinnati, Ohio). Prior to use, 2.9×10^4 BG spores were added to each liter of metalworking fluid. About 300 L/min of filtered air was supplied to the simulator. The ESP and BioSampler sampled from the exhaust flow, which was then passed through a filter bank.

In all the experiments, the microbial concentrations measured by the electrostatic precipitator were compared to those measured by the BioSampler, which was operated at a flow rate of 12 L/min and was flow-calibrated by a Buck calibrator. The collection medium in the BioSampler was 20 mL of deionized and sterilized water, without any wetting agent.

In the chamber experiments and in the field experiments in an office, an aliquot of 5 mL from the BioSampler was filtered through Nuclepore polycarbonate track-etch membranes (47 mm diameter, 0.4 μm pore size, Fisher Scientific); each filter membrane was then placed onto agar and incubated. The airborne concentration of microorganisms in animal barns have previously been reported to be very high, about 10^5–10^7 spores/m^3 in Finnish cow barns.[16] Therefore, in the horse barn experiments, only a 100 μL aliquot of suspension from the BioSampler was spread directly on agar and incubated.

In all experiments, to cultivate the collected bacteria, we used tryptic soy agar (40 g/L, Becton Dickinson and Co., Sparks, Md.) supplemented with cycloheximide to inhibit the growth of fungi (0.5 g/L, Sigma-Aldrich Co., St. Louis, Mo.). To cultivate the fungi, we used malt extract agar (15 g/L, Becton Dickinson and Co.) supplemented with streptomycin sulfate to prevent the growth of bacteria (40 mg/L, Sigma-Aldrich Co.). For enumerations of the BG spores , the agar plates were incubated for only one day at 25°C because of colony overgrowth, while for all other bacteria the incubation time was between 2 to 4 days. For fungi, the incubation time was at least 4 days at 25°C. After incubation, the colonies were counted with a colony counter (Scienceware Electronic Handheld Colony Counter, Fisher Scientific).

Experimental Procedures

Before performing any experiments in the walk-in chamber, the chamber was ventilated with clean air until OPC1 indicated zero background readings in the 0.3 μm to 5 μm particle size range. The dust and microorganisms from the carpet were dispersed by moving the vacuum cleaner nozzle over the carpet forward and backward five times. The vacuum cleaner was operated in reverse airflow by connecting the canister exhaust to the nozzle. For each experiment, a piece of carpet (0.23 m wide, 0.84 m long) was cut from a larger carpet that had been used and soiled in a residence for several years. Sampling with the ESP and BioSampler were initiated when OPC1 indicated a steady aerosol concentration in the chamber.

For each experimental condition, the overall physical collection efficiency of the ESP, $E_{OVERALL}$, was determined by comparing the total aerosol concentration in the first 10 channels of OPC2, C_{DOWN}, with that of OPC1, C_{UP}:

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

Because electrically charged particles are removed by the electric field in the ESP to either the upper or lower (agar) electrode, depending on the amount and polarity of electrical charges on the airborne particles, the overall physical collection efficiency at various precipitation voltages is an indicator of the number of charges on the airborne particles. The overall physical collection efficiency was determined every 30 minutes for 2 hours at $V_{ESP} = \pm 300$ V, ± 1000 V, and ± 3000 V for two different conditions of vacuum cleaning: with and without beater bar activation. It was expected that the continuous rubbing of the beater bar brushes on the carpet would result in more highly charged aerosolized particles than through removal of carpet-embedded particles by airflow alone.

To further study the effect of electrical charges on airborne particles, we also performed experiments with positive and with negative ionization in the ESP's inlet section. Previous laboratory tests conducted with positive ionization in the inlet section had shown that the precipitation voltage applied to the electrode above the agar plates needs to be positive to collect the microorganisms on the grounded agar plates.[17] Therefore, the precipitation voltages were positive at +300 V, +1000 V, and +3000 V for the tests with positive ionization. For these tests with carpet dispersed microorganisms and dust, the ionization voltages, $V_{Charging}$, were 1.1 V, 1.3 V, and 1.5 V, which resulted in ionization currents of 90 mA, 110 mA, and 120 mA, respectively. We also had negative ionizers made for our experiments (Wein Products, Inc.). Ionization voltages of 0 V, −1.5 V (20 mA), −2.0 V (30 mA), −2.5 V (40 mA), and −3.0 V (50 mA) were used at negative precipitation voltages of −300 V, −1000 V, and −3000 V.

Because we had seen through previous experiments[12] that the high initial electrical charges on laboratory-generated aerosols is reduced in time by naturally present ambient radiation, we also measured the overall physical collection efficiency as a function of time for microorganisms and dust particles dispersed from carpets. The magnitude of the collection efficiency measured at positive and negative precipitation voltages (without ionization in the inlet section) reflects the amount of positive or negative electrical charges on the microorganisms, as the collection of microorganisms in the ESP depends on the amount and polarity of charges on the microorganisms and on the precipitation voltage applied.

The relative biological collection efficiency of the ESP, $E_{Rel\ Biol}$, was determined by comparing the number of colony forming units per liter of air (CFU/L) sampled by the ESP, $(C_{CFU/L})_{ESP}$, with the CFU/L enumerated from sampling with the BioSampler, $(C_{CFU/L})_{BioSampler}$:

$$E_{Rel\,Biol} = (C_{CFU/L})_{ESP}/(C_{CFU/L})_{BioSampler} \quad \textbf{(2)}$$

This procedure for determining the relative biological collection efficiency of the ESP was used in all experiments. The BioSampler was used as a reference sampler. No bioaerosol

sampler is 100% efficient. The efficiency of the BioSampler compares well with that of other reference samplers.[9]

When dust and microorganisms were dispersed from carpets, a significant reduction in charge was found over a period of a few minutes. Thus, the ESP's collection efficiency decreased significantly in a few minutes, and only a small number of CFUs were enumerated on the agar plates in the ESP. To have a sufficient aerosol concentration for the determination of the ESP's biological collection efficiency, we prepared a 20 mL suspension of BG spores at a liquid concentration of 1.2×10^6/mL and continuously dispersed the microorganism suspension through the collison nebulizer into the chamber. Using BG spores in these experiments had the additional feature of simulating *B. anthracis* spores. The ESP sampled at 4 L/min for 2 minutes, while the BioSampler sampled at 12 L/min for 7 minutes.

In the office experiment, four adults sat around a small table in a faculty office, with the door closed, where they chatted with each other, coughed occasionally, and every 2 minutes rubbed their skin. The electrostatic precipitator and BioSampler were located in the middle of the table and were operated for 15 minutes at 4 L/min and 12 L/min, respectively. The experiments were performed at precipitation voltages of ±3000 V without any charging in the ESP's inlet section.

In the horse barn experiment, the bioaerosol concentrations were expected to be higher;[16] therefore, the sampling time was reduced to 5 min for both devices operated at the same flow rates as in the office experiment. The first experiments were again performed at precipitation voltages of ±3000 V without any charging in the ESP's inlet section. Subsequent experiments were performed at precipitation voltages of +1000 V and +3000 V with positive ionization at 1.1 V (90 mA). Ionization was added to the ESP in some experiments to experimentally determine how easily and to what extent electrical charges can be added to the airborne microorganisms. In these experiments, the biological evaluations were not limited to bacteria, but were also performed for fungi.

In the metalworking fluid experiment, a 3.8 cm diameter metal rod was rotated at 6000 rpm, and the liquid pump ejected the metalworking fluid through a nozzle against the rotating rod at a flow rate of 450 mL/min. When the Grimm particle size spectrometer indicated a stable aerosol concentration in the enclosure surrounding the rotating rod, the ESP (4 L/min) and the BioSampler (12 L/min) took samples for 1 min each at several precipitation voltages, with and without positive or negative ionization.

Throughout this study, each experiment for a specific condition in the field environments or in the controlled environments simulating field conditions was repeated at least three times.

Data Analysis

The data analysis was performed using analysis of variance (ANOVA) available as SAS version 8.0.[18] *P* values of <0.05 were considered significant. The model used for this experiment was a 2-way ANOVA. The independent variables were charging voltage and collection voltage. The dependent variable was overall physical collection efficiency or relative biological collection efficiency. The 2-way ANOVA with two factors was performed by using PROC GLM program statements with SAS. We also used the Dunnett and SNK methods for comparisons among the different charging and collection voltages.[19] The data displayed in the figures represent the means and standard deviations of at least three repeats.

RESULTS AND DISCUSSION

Figure 2 shows the overall physical collection efficiency of the new electrostatic precipitator as a function of particle size (0.3 μm–5 μm) for microorganisms and dust particles dispersed from soiled carpets in the walk-in chamber. Because the overall physical collection efficiency is determined from measurements of the aerosol concentrations exiting the ESP relative to those entering the ESP, as measured by the two Grimm optical particle counters, the data do not specify where the particles are collected inside the ESP. The indicated particle diameters, d_p, are optical equivalent particle diameters.[20] For measurements in which the measured quantity does not change much with particle size, as in Figure 2, it is not important which measurable index is used to characterize the particle size (e.g., optical vs. aerodynamic).

During the measurements, no charging was applied to the aerosolized particles in the inlet of the ESP. When the vacuum cleaner nozzle dispersed carpet particles without any beater bar rotation (Figures 2a and 2c), the overall physical collection efficiency depended strongly on the precipitation voltage. While increasing slightly with particle size, the overall physical collection efficiency ranged from about 70 to 85% at positive (Figure 2a) or negative (Figure 2c) precipitation voltages of 3000 V. Correspondingly, the overall physical collection efficiency was 50 to 65% at $V_{ESP} = \pm 1000$ V, and 15 to 35% at $V_{ESP} = \pm 300$ V. Such a strong dependence on precipitation voltage is possible only if the particles carry electric charges that create migration velocities toward the electrodes in the ESP, which are located perpendicular to the airflow. The wide range of collection efficiencies measured at the three different precipitation voltages indicates a wide range of electric charges carried by the airborne microorganisms. Airborne microorganisms carrying several electric charges are collected by a low precipitation voltage, while those carrying few electric charges require a stronger electric field for collection.

Thus, these data are an indirect, nonquantified measure of the high electrical charge on microorganisms and dust particles dispersed into the air by airflow over contaminated carpets. Because the data in Figure 2 show the overall physical collection efficiency, the data for positive precipitation voltages are similar to those for negative precipitation voltages. An overall physical collection efficiency of 100% for either positive or negative precipitation voltage would indicate that all the airborne microorganisms carry electric charges of a magnitude that is high enough for them to be attracted to the precipitation

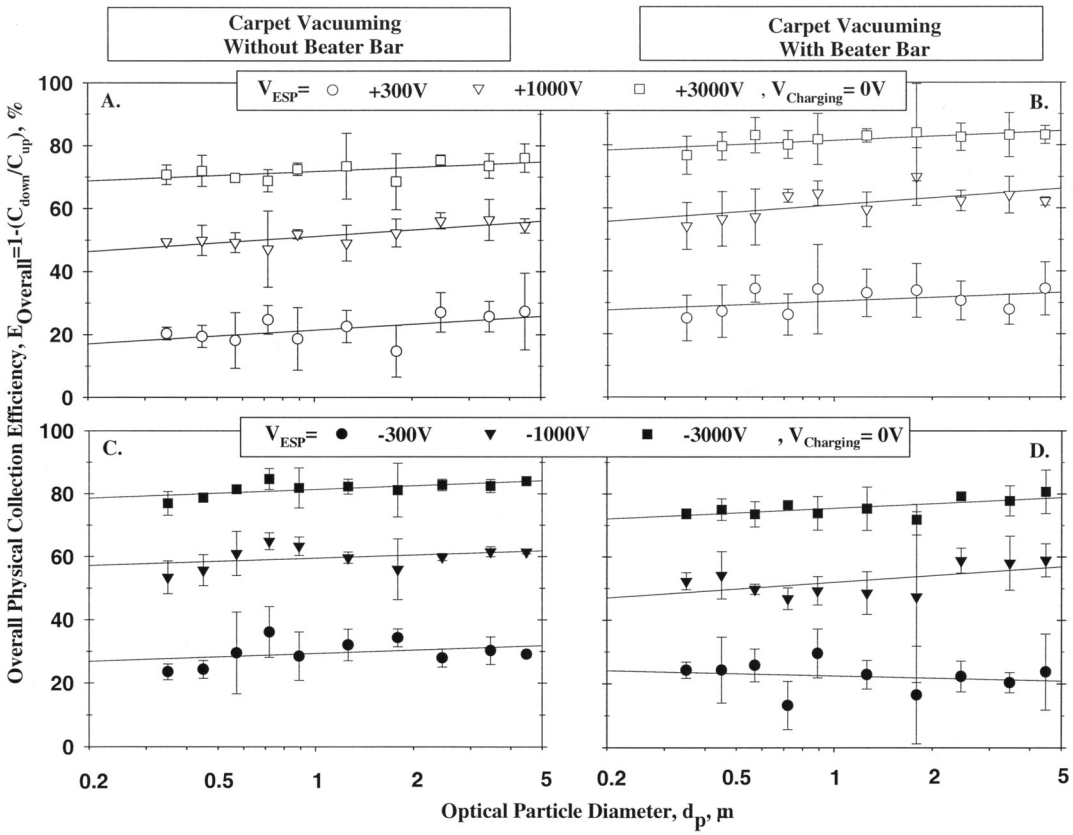

FIGURE 2. The overall physical collection efficiency of the new electrostatic precipitator at 4 L/min as a function of particle size, showing the effect of high electrical charge transferred to aerosolized microorganisms and dust particles, when dispersed from dirty carpets. The particles were dispersed in the walk-in chamber for 2 min, followed by 1 min of mixing in the chamber before sampling with Grimm particle size spectrometers for 1 min.

surfaces in the ESP. Any particle that deposits on the agar surface at the bottom of the ESP, while a precipitation voltage of positive polarity is applied, will deposit on the upper electrode plate, when the polarity is reversed to negative precipitation voltage. When the biological efficiency is measured (see below), the data indicate what shows up only on the agar plates and grows into colonies. All of these data reflect the polarity and strength of electrical charges on the sampled airborne microorganisms.

We had expected that the action of the beater bar, that is, the rubbing of the bristles over the carpet surface, would significantly increase the charge on the aerosolized particles. However, Figures 2b and 2d show that the overall physical collection efficiencies were approximately the same with beater bar action as they were without such rubbing action. Thus the electrification of the dispersed particles appears to be primarily due to contact charging resulting from differences in the electrochemical potential between the particles and the contacting surface.[21–23] Figure 2 also suggests that the new ESP is quite effective in removing aerosolized particles from the air to the inner surfaces of the ESP without any charging in its inlet section. It should be confirmed by more data under different conditions that ESP can efficiently collect airborne microorganisms without a charge application in its inlet section. Sampling would be simplified if airborne microorganisms were not subjected to stresses and ozone generated during electrical charging in the inlet section.

Because the overall physical collection efficiency for the ESP was quite satisfactory at precipitation voltages of ±3000 V without imparting additional electrical charges onto the airborne particles entering the ESP, we wondered whether the high electrical charges on the particles would remain high with time. Figure 3a shows that the overall physical collection efficiency of the ESP (averaged over the size range between 0.3 and 5 μm, Q = 4 L/min) for carpet-dispersed particles without beater bar action is reduced to less than half of its initial value after an aging time, t_{aging}, of 2 hours, that is, the electrical charges on the dispersed particles were significantly reduced with time after aerosolization. The data for particle dispersion with beater bar action (Figure 3b) are similar. Charge reduction has also been observed in aging experiments with *P. fluorescens* vegetative cells.[17] The high charges are reduced by attachment of positive and negative ions that are naturally present in the atmosphere.

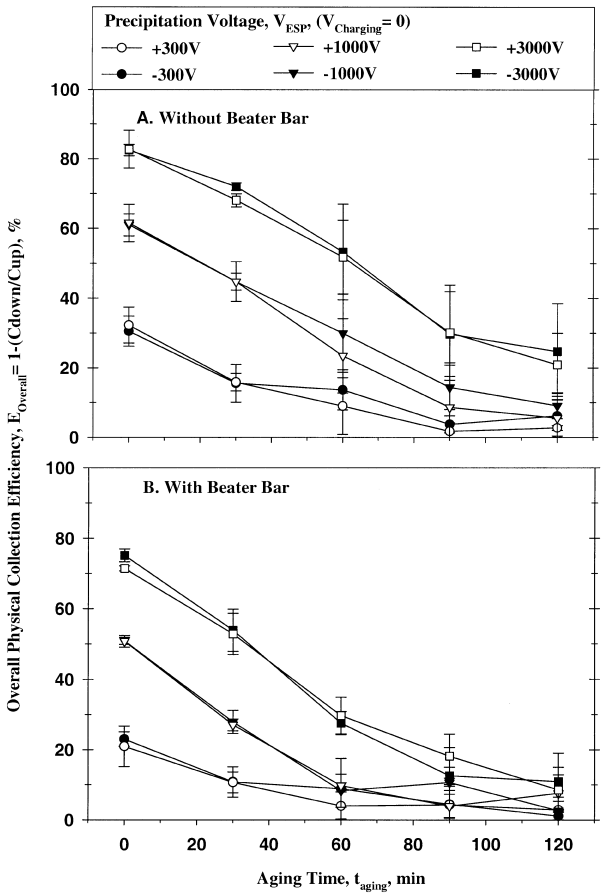

FIGURE 3. The effect of time in decreasing the electrostatic charge on carpet-dispersed microorganisms and dust particles

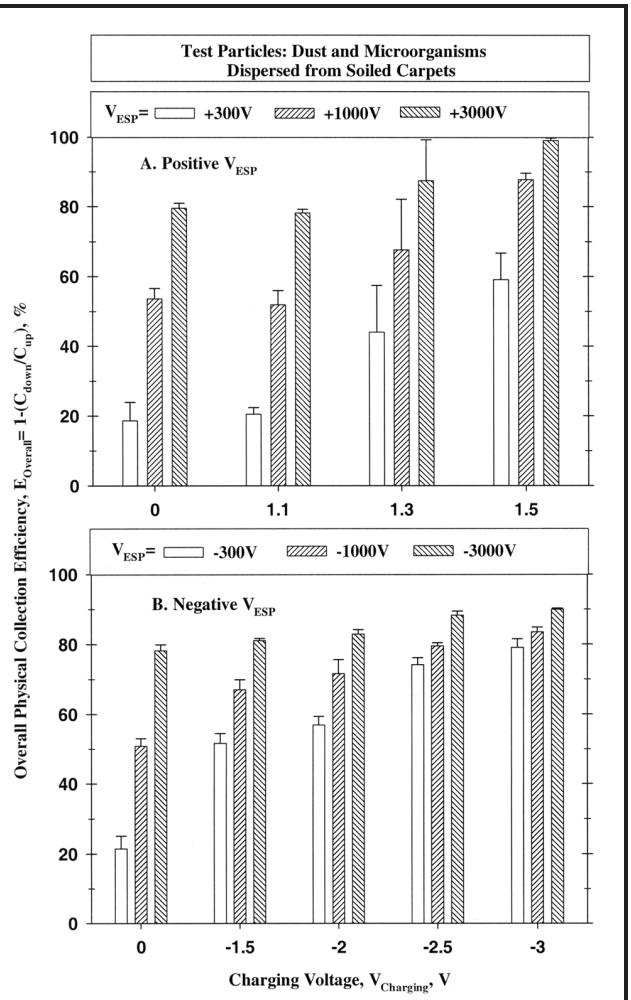

FIGURE 4. The effect of electrical charging on the collection of airborne microorganisms and dust particles dispersed from soiled carpets. No beater bar action. $Q_{ESP} = 4$ L/min.

Thus, our first conclusion is that during the first few minutes after their dispersal, particles may carry very high electric charges. While not exclusively proven by our experiments, the physics of particle dispersion[21–23] suggests that most aerosolization processes for microorganisms, dust particles, and powders are likely to result in high electric charges, be it an involuntary release (e.g., during manufacturing) or an intended release (e.g., during an act of bioterrorism).

Waterborne bacteria have been shown to naturally contain electrical charges in their cell membranes.[24–26] The amount of electrical charges naturally contained in the membranes of airborne microorganisms is still unknown. We were curious to what extent electrical charges can be added to airborne microorganisms, and, therefore, performed experiments with microorganisms dispersed from carpets using the ESP with the ionizers in its inlet section turned on, and operating at different charging levels.

Figure 4a shows that the overall physical collection efficiency at the low precipitation voltage of 300 V is significantly increased when the positive ionizer in the ESP inlet is 1.3 V (110 mA) or higher, that is, electrical charges can be added to the airborne microorganisms. Our prior experiments with laboratory-generated *P. fluorescens* cells and BG endospores indicated a net negative charge on the dispersed cloud of microorganisms.[13] Thus, it is not surprising that the overall physical collection efficiency at a negative precipitation voltage of −300 V increased to higher levels when the negative ionizer operated at a threshold level of −1.5 V (20 mA) or more (Figure 4b). Analyzing the data with ANOVA confirmed that the overall physical collection efficiency depends on the precipitation voltage ($p < 0.0001$) and on the charging voltage above the indicated threshold values ($p < 0.0001$).

Because Figure 3 shows a significant reduction in the overall physical collection efficiency of the ESP several minutes after particles are dispersed from the carpets, the biological collection efficiency was determined for a steady aerosol concentration of BG endospores, dispersed by a collison nebulizer in the walk-in chamber. Figure 5 shows the CFU count measured on the ESP agar plates relative to the comparable CFU count enumerated from plating out the collection liquid of the BioSampler. This relative biological collection efficiency of the ESP is on the order of 20%, which is further explained below.

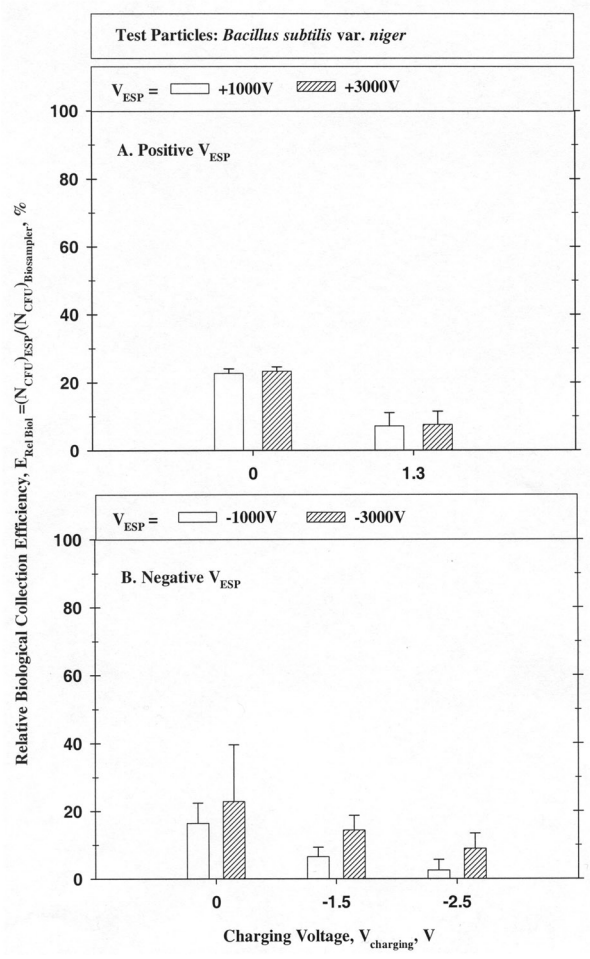

FIGURE 5. The CFU count on the agar plates at the bottom of the ESP relative to the CFU count determined by parallel sampling with the BioSampler. The *Bacillus anthracis* simulating BG endospores were dispersed through a Collison nebulizer in the walk-in chamber. $Q_{ESP} = 4$ L/min, $Q_{BioSampler} = 12$ L/min.

Assessment of the electrical charges on airborne microorganisms by collection in the ESP is facilitated by reference to the BioSampler, because the BioSampler collects the airborne microorganisms in a liquid without use of any electric fields. As indicated above, over 95% of the BG endospores were monodisperse; thus, the low biological collection efficiencies in Figure 5 cannot be attributed to the breakup of clumped microorganisms in the BioSampler.

Statistical analysis with ANOVA indicated that for positive precipitation voltages (Figure 5a), the relative biological collection efficiency depended on whether the microorganisms were charged or not ($p < 0.0001$), but not on the level of the precipitation voltages used in these tests ($p = 0.6802 > 0.05$). For negative charging (Figure 5b), the ANOVA output also showed that there was a statistically significant difference between charging and no charging ($p = 0.0157 < 0.05$), but no difference between the tested levels of precipitation voltage ($p = 0.0639 > 0.05$). Comparison tests, using the Dunnett and SNK methods, showed no statistically significant difference between the charging voltages of -1.5 V and -2.5 V.

The overall physical collection efficiency data (Figure 4) demonstrated an increase with increased charging above a threshold. However, the relative biological collection efficiency decreased (Figure 5). The reason is that, with the present design of the ESP, the highly charged microorganisms are deposited on inner surfaces in the ESP before reaching the agar plates. This was visually confirmed, as more microorganisms were enumerated at the leading edge of the first agar plate with charging than without charging. This suggests that future design improvements should place the ionizer closer to or above the leading edge of the first agar plate.

Another reason for the decrease in biological collection efficiency may be that the electrical charging puts a burden onto the membranes of the bacterial cells and thus decreases their microbial viability.[13] When particles are excessively charged, the repulsive forces among the particles may also direct some of them to deposition surfaces other than the agar plates. In summary, our tests with laboratory-dispersed microorganisms suggest that electrical charges can be added to the already existing charges on airborne microorganisms, but the process of charging by ionization may damage or kill the microorganisms, depending on their sensitivity level.

Figure 6a shows the ESP's relative biological collection efficiency measured with BG endospores in the walk-in chamber. The figure compares the efficiency measured at a precipitation voltage of $+3000$ V with the efficiency measured at -3000 V, without any charging in the ESP's inlet. Figure 6b shows the data obtained in the faculty office with four adults talking, coughing, and occasionally rubbing their skin, while the ESP sampled without additional charging. Statistical analysis showed no significant differences in the biological collection efficiency data between the negative and positive precipitation voltages for the test chamber experiment ($p = 0.5293 > 0.05$) and the office experiment ($p = 0.1695 > 0.05$).

However, the efficiency data for the negative precipitation voltage appear to be slightly higher in the faculty office. This would again indicate a net negative charge on the dispersed cloud of microorganisms in natural environments. From an ESP design point of view, this suggests that agar plates should be considered on both electrodes of the ESP so that negatively and positively charged microorganisms can be enumerated at the same time. Differentiation between positively and negatively charged microorganisms would offer an additional signature in the characterization of airborne microorganisms. The biological collection efficiency in the small office (Figure 6b) may have been affected by lack of monodispersity of the microorganisms and by skin flakes containing many microorganism (each flake registered as one microorganisms in the ESP, but potentially as more than one by the BioSampler, if deagglomerated during collection in the BioSampler liquid).

Figure 7 partially explains why the biological collection efficiencies in Figures 5 and 6 were so low. Because all the ESP data presented in Figures 5 and 6 are related to the biological collection efficiency of the BioSampler, Figure 7 assumes for

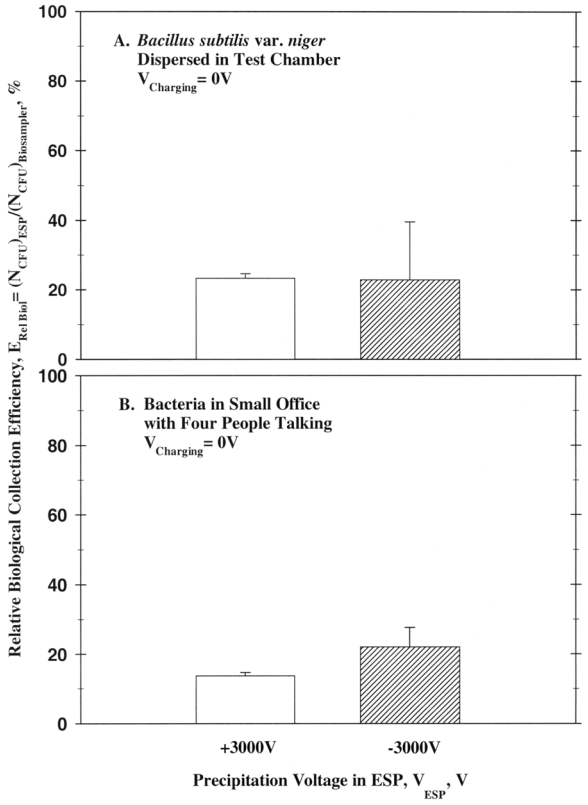

FIGURE 6. The relative biological collection efficiency of the ESP in the chamber and office experiments, indicating that positively and negatively charged microorganisms can be differentiated by placing agar plates on both electrodes in the ESP. Q_{ESP} = 4 L/min, $Q_{BioSampler}$ = 12 L/min.

this illustration a 100% biological collection efficiency for the BioSampler. When sampled with the ESP at a positive precipitation voltage of +3000 V, about 23% of the CFUs were enumerated on the tryptic soy agar (TSA) plates. An additional 23% were collected at a negative precipitation voltage of −3,000 V. Thus, about 46% of the microorganisms enumerated in the liquid of the BioSampler were also enumerated on the agar plates in the ESP, that is, 46% of the microorganisms carried sufficient electrical charges to be collected on the agar plates in the ESP and be enumerated as colonies.

However, the overall physical collection efficiency of the ESP was measured to be about 80% for these conditions (Figure 4). Thus, 34% of the microorganisms were collected inside the ESP but were not recognized as colonies on the agar surfaces. One possible explanation for this discrepancy is that the electrostatic field between the two electrodes bulges outward at its edges. Because the electrodes in our ESP were of the same length as the agar plates, it is likely that many of the highly charged microorganisms were affected by the bulging electrostatic field and were deposited onto the inner wall of the ESP ahead of the leading edge of the first agar plate. Improvement in the ESP design should reduce the losses inside the ESP. The remaining 20% are particles that were either neutral or had insufficiently high charges for collection in the ESP, as presently designed.

Because our tests in the walk-in chamber and in the office produced meaningful results, the ESP and BioSampler were taken to a horse barn for further testing. At this site we measured not only the airborne concentrations of bacteria, but also those of fungal spores. As seen in Figure 8a, the relative biological efficiencies were higher at the negative precipitation voltage than at the positive precipitation voltage, for both

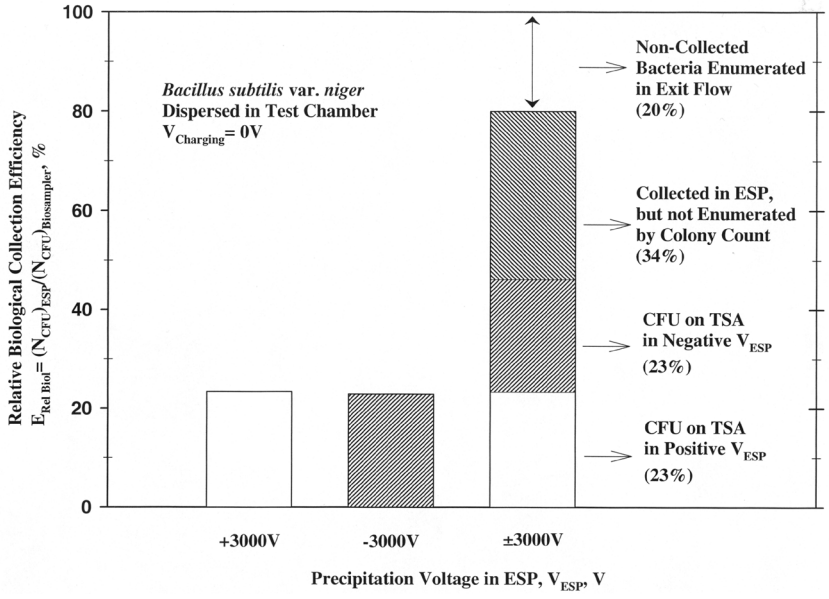

FIGURE 7. Assessment of the enumerated and nonenumerated microorganisms, when sampled with an electrostatic precipitator

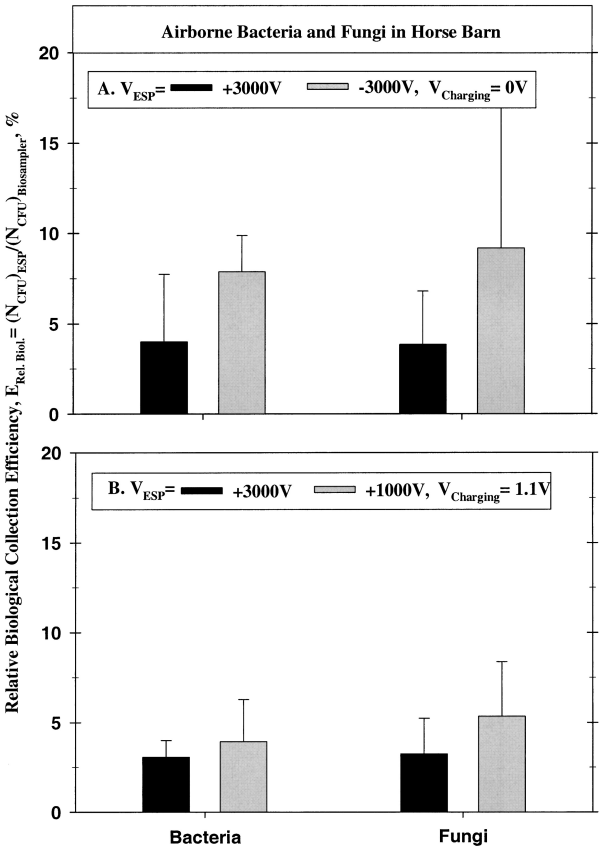

FIGURE 8. Positive versus negative charges of airborne bacteria and fungi in a horse barn, measured by the ESP. $Q_{ESP} = 4$ L/min, $Q_{BioSampler} = 12$ L/min.

bacteria and fungi. Thus we have found that the net charge on the assembly of airborne microorganisms in most of our laboratory and field tests is negative.

Visual observation of the agar plates indicated that most of the bacteria were deposited on the first agar plate, while the fungi were quite homogeneously distributed on the second agar plate. We conclude that the bacteria, due to their size below about 1 μm, have high electrical mobility and therefore migrate to the first agar plate, while the fungal spores of 2 to 3 μm have higher inertia relative to their electrical charge and are therefore collected on the second plate or deposited behind the plates when exposed to the same collection conditions.

The relative biological collection efficiencies in the horse barn experiments were lower than in our prior experiments. The bacteria and fungal spores were analyzed separately: one set of agar plates was analyzed for fungi and one for bacteria. We observed that some of the species of fungi and bacteria inhibited the growth of other species of fungi and bacteria. Also, some species of fungi and bacteria grew much faster than other species and became the dominant species. Because there were many more CFUs on the ESP plates than on the plates with a much-diluted collection liquid from the BioSampler, we attribute the lower collection efficiency values for the ESP plates to growth inhibition on the ESP plates. The fungi may have been more monodisperse than the bacteria in the horse barn, which would result in the higher biological collection efficiencies for fungi, as seen in Figure 8.

We also experimented with positive charging in the ESP inlet, while sampling in the same horse barn (Figure 8b). All relative biological collection efficiencies were proportionally reduced, similar to our tests in the walk-in chamber. It is expected that future design improvements will result in higher efficiencies.

Figure 9 shows the data obtained from sampling in the metalworking simulator. In these experiments, the metalworking fluid contained BG endospores. When no charging was applied to the ESP, the relative biological collection efficiency was about the same for positive and negative precipitation voltages. Increasing the precipitation voltage from 1000 V to 3000 V,

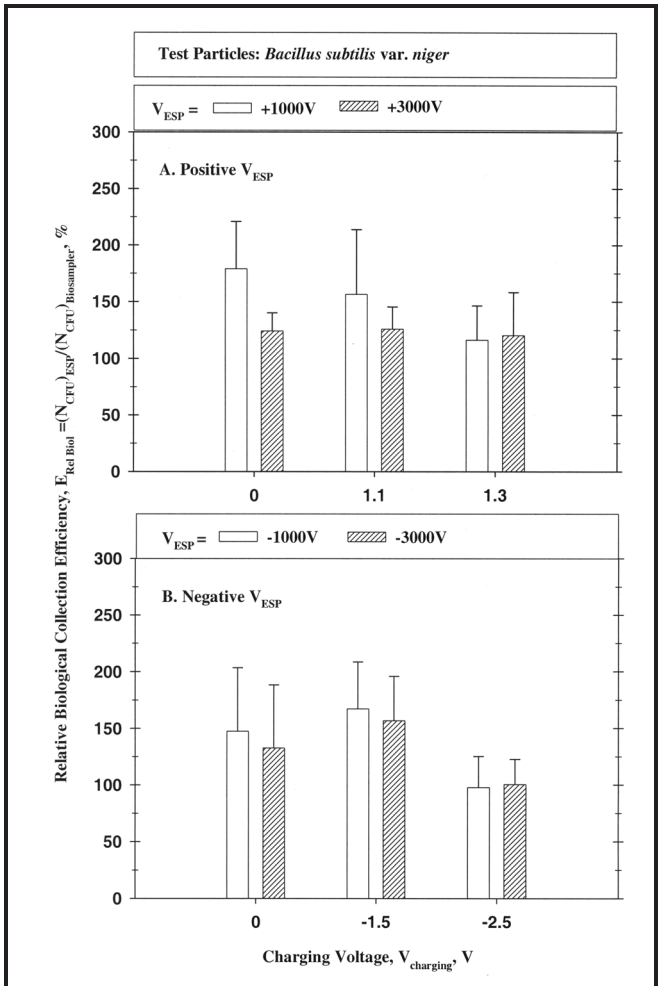

FIGURE 9. Tests of the ESP's relative biological collection efficiency in a facility simulating operations with metalworking fluids. $Q_{ESP} = 4$ L/min, $Q_{BioSampler} = 12$ L/min.

positive or negative, somewhat decreased the collection efficiency. Charging in the inlet did not significantly change the efficiency values, as seen visually in Figure 9, and also verified by statistical analysis.

The relative biological collection efficiencies were above 100%, considerably higher than in all of the other experiments. We believe that the high collection efficiency was caused by the high turbulence in the air surrounding the cutting fluid operation, which was still quite high when the aerosol entered the ESP. Thus, turbulent deposition rather than electrostatic forces may have caused the high relative biological collection efficiencies and the lack of differentiation between collection efficiencies for different sampling parameters. The high turbulence may also have affected the sampling by the BioSampler, although the inlet flows were calculated to be approximately isokinetic.

Calculations for isokinesis assume laminar flow and are not valid for turbulent flow, which has velocity components perpendicular to the main flow, thus increasing particle deposition.[15] This suggests that future design modifications for the ESP should consider a narrow inlet slot with a modest pressure drop so that the turbulence scale and intensity is reduced, and the aerosol is uniformly distributed perpendicular to the flow direction.

CONCLUSIONS

Our experimental data show that in most of the tested environments the net charge on the airborne microorganisms was negative. The new electrostatic precipitator is capable of differentiating positively from negatively charged bacteria and fungi. The data and visual observations of the deposits in the ESP suggest that agar surfaces or other substrates should be considered on the positive as well as the negative electrodes so that the positively and negatively charged microorganisms can be collected at the same time and can be differentiated from each other. The amount of electrical charge on microorganisms of similar size is differentiated by differences in the collection efficiency at different precipitation voltages.

In our tests, aerosolized microorganisms were collected without additional charging in the ESP inlet, if they were collected within minutes of their dispersal. With time, the electrical charges on the microorganisms are reduced to their natural charge level, which depends on the microorganism's cell structure.

The ESP's ability to differentiate positively from negatively charged microorganisms adds a signature to the sampled microorganisms that may assist in their identification or differentiation. Because the ESP is essentially an open channel, low power is required for the sampling flow through the ESP. Also, very little power is needed for creating the precipitation voltage across the electrodes. Thus, this method is worth pursuing further for low power monitoring of airborne microorganisms and their electric charge distributions, e.g., in an antibioterrorism network.

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