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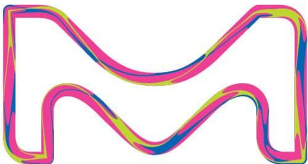

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Effect of Electrical Charges and Fields on Injury and Viability of Airborne Bacteria

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Abstract: In this study, the effects of the electric charges and fields on the viability of airborne microorganisms were investigated. The electric charges of different magnitude and polarity were imparted on airborne microbial cells by a means of induction charging. The airborne microorganisms carrying different electric charge levels were then extracted by an electric mobility analyzer and collected using a microbial sampler. It was found that the viability of *Pseudomonas fluorescens* bacteria, used as a model for sensitive bacteria, carrying a net charge from 4100 negative to 30 positive elementary charges ranged between 40% and 60%; the viability of the cells carrying >2700 positive charges was below 1.5%. In contrast, the viability of the stress-resistant spores of *Bacillus subtilis* var. *niger* (used as simulant of anthrax-causing *Bacillus anthracis* spores when testing bioaerosol sensors in various studies), was not affected by the amount of electric charges on the spores. Because bacterial cells depend on their membrane potential for basic metabolic activities, drastic changes occurring in the membrane potential during aerosolization and the local electric fields induced by the imposed charges appeared to affect the sensitive cells' viability. These findings facilitate applications of electric charging for environmental control purposes involving sterilization of bacterial cells by imposing high electric charges on them. The findings from this study can also be used in the development of new bioaerosol sampling methods based on electrostatic principles. © 2002 Wiley Periodicals, Inc. *Biotechnol Bioeng* **79**: 229–241, 2002.
Keywords: charging of airborne microorganisms; electrical field; *P. fluorescens*; *B. subtilis*; relative recovery

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INTRODUCTION

The goal of this study was to determine how the magnitude and polarity of electric charges affect the viability of airborne bacteria. Such information may be used to develop new techniques for measurement and control (i.e., inactivation or sterilization) of airborne microorganisms. Most commonly used methods for measurement of airborne bacteria, impaction and impingement, are known to impart significant mechanical stress on sensitive microorganisms (Lin et al., 2000; Stewart et al., 1995; Terzieva et al., 1996), which may lead to underestimations of the bioaerosol concentration. One of the methods showing potential for being a less stressful bioaerosol collection technique is electrostatic precipitation. In this method, the airborne particles are electrically charged and then subjected to an electric field. The electric field forces remove the electrically charged particles from the air stream and deposit them onto a collection substrate. When electrostatic forces collect particles, their velocity component perpendicular to the collection medium is about two to four orders of magnitude lower than that in bioaerosol impactors and impingers operated at comparable sampling flow rates. Thus, this study was undertaken as a part of our research on development and evaluation of a new bioaerosol sampling method utilizing electrostatic precipitation. Mainelis et al. (1999) modified a commercially available electrostatic aerosol sampler (Model 3100, TSI, Inc., St. Paul, MN) and used it to collect airborne bacteria. This bioaerosol collector efficiently removed most of the airborne bacteria from the air stream, but it inactivated sensitive vegetative cells, such as *Pseudomonas fluorescens*. These effects were attributed to the corona discharge, which is the charging mechanism in conventional electrostatic precipitators.

Because studies on water-borne microorganisms have indicated that cells in a liquid may carry thousands of elementary charge units (Sherbet and Lakshmi, 1973), we expected that microorganisms in the airborne state

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may also be electrically charged and that their collection by electrostatic forces is possible without prior charging. As a step toward investigating this hypothesis, we developed and validated a new experimental setup for studying the electrobiological properties of airborne microorganisms. In this setup, a new bioaerosol generator aerosolizes the microorganisms and electrically charges them by induction; a new electrical mobility analyzer then extracts microorganisms of specific charge ranges (Mainelis et al., 2002). Using this setup, we measured the electric charges on biological and nonbiological airborne particles and investigated some of the parameters affecting the magnitude of such charges (Mainelis, et al., 2001). This study has shown that airborne vegetative cells of *P. fluorescens* and endospores of *Bacillus subtilis* var. *niger* have a net negative electric charge. We also found that the magnitude of the electric charge carried by individual cell subpopulations depends on the dispersion method and that individual cell subpopulations carry as many as 13,000 elementary electric charges. This finding contrasts with the low electric charges carried by aerosolized NaCl particles of the same size. On the one hand, airborne biological particles carrying such a high electric charge can be effectively collected by electrostatic forces without any additional charging. On the other hand, such high charges may render microorganisms unviable, which could be utilized for disinfection purposes. It was also concluded that the amount of electric charge carried by aerosolized bacteria could be used as an indicator of the mechanical stress endured by them during aerosolization from deionized water. Additional experiments simulating the effects of radiation, which is naturally present in the environment, have shown that atmospheric radiation may significantly reduce the amount of electrical charge carried by airborne microorganisms (Mainelis et al., 2001). Thus, microorganisms that have been airborne for some time may have to be electrically charged before collection by electrostatic forces. However, the electric charge externally imparted on the microorganisms may be drastically different from the natural charge carried by them, possibly affecting their viability. Thus, the goal of this study was to determine how the magnitude and polarity of electric charges that are externally imparted affect the viability of airborne bacteria. An experimental setup developed earlier (Mainelis et al., 2002) was used for this purpose.

In this study we tested two bacterial species. Vegetative cells of the Gram-negative bacterium *Pseudomonas fluorescens* are commonly found in ambient air (Górny and Dutkiewicz, 1998; Nevalainen, 1989) and were selected to represent sensitive bacteria (Neidhardt et al., 1990). When suspended in liquid, cells of this microorganism carry a negative electric charge (Yamane et al., 1970). Spores of the Gram-positive bacterium *Bacillus subtilis* var. *niger* (BG) are known to be very resistant to many adverse conditions (Sneath, 1986), and were thus selected to represent stress-resistant strains. BG spores suspended in liquid are also negatively charged (Tochikubo et al., 1975).

MATERIALS AND METHODS

Experimental Setup for Induction Charging and Collection of Electrically Charged Microorganisms

The effect of electrical charges on the viability of microorganisms in the airborne state was studied using the experimental setup shown schematically in Figure 1. This setup has been discussed in detail elsewhere (Mainelis et al., 2002), and here we present only its main features.

The experimental setup consists of two principal components: the bioaerosol generator with charge induction and the electrical mobility analyzer coupled with an optical particle counter and a microbial sampler. In the bioaerosol generator, the microorganisms are aerosolized through a single orifice in the center stem of a Collison nebulizer (BGI, Inc., Waltham, MA). Passage of the nebulizer airflow, $Q_{NEB} = 1.2$ L/min, through the orifice creates a negative pressure that pulls the suspension feed into the air jet exiting from the orifice. This suspension is then broken up by the air jet into a dispersion of droplets of very wide size distribution (May, 1973). The droplets pass through the induction ring positioned 9 mm from the Collison orifice. The ring has an inner diameter of 37 mm and its plane is oriented perpendicular to the orifice axis. Application of a positive induction voltage, $V_{INDUCTION}$, to the stainless-steel Collison stem (while the induction ring is grounded) creates an electric field in the axial direction that induces positive electric charges onto the droplets as they are being formed from the liquid exiting the Collison stem. When a negative voltage is applied to the Collison stem, negative charges are induced onto the droplets. As the droplets pass through the induction ring, they are surrounded by dry, clean airflow, $Q_{DRY1} = 50$ L/min, which separates the droplets from each other and starts drying them. After the liquid content of each charged droplet containing a microorganism has evaporated, the electric charge remains on the microorganism. The largest droplets settle to the bottom of the disperser and are drained. Addition of more dry air, $Q_{DRY2} = 30$ L/min, completes the desiccation of the droplets so that only charged airborne microorganisms and much smaller droplet residues leave the aerosol generator. By increasing or decreasing the positive or negative charging voltage, $V_{INDUCTION}$, between the grounded induction ring and the Collison stem, we manipulate the magnitude and polarity of electric charges imposed onto the airborne microorganisms. The charging voltage is applied through an external power source (DC Power

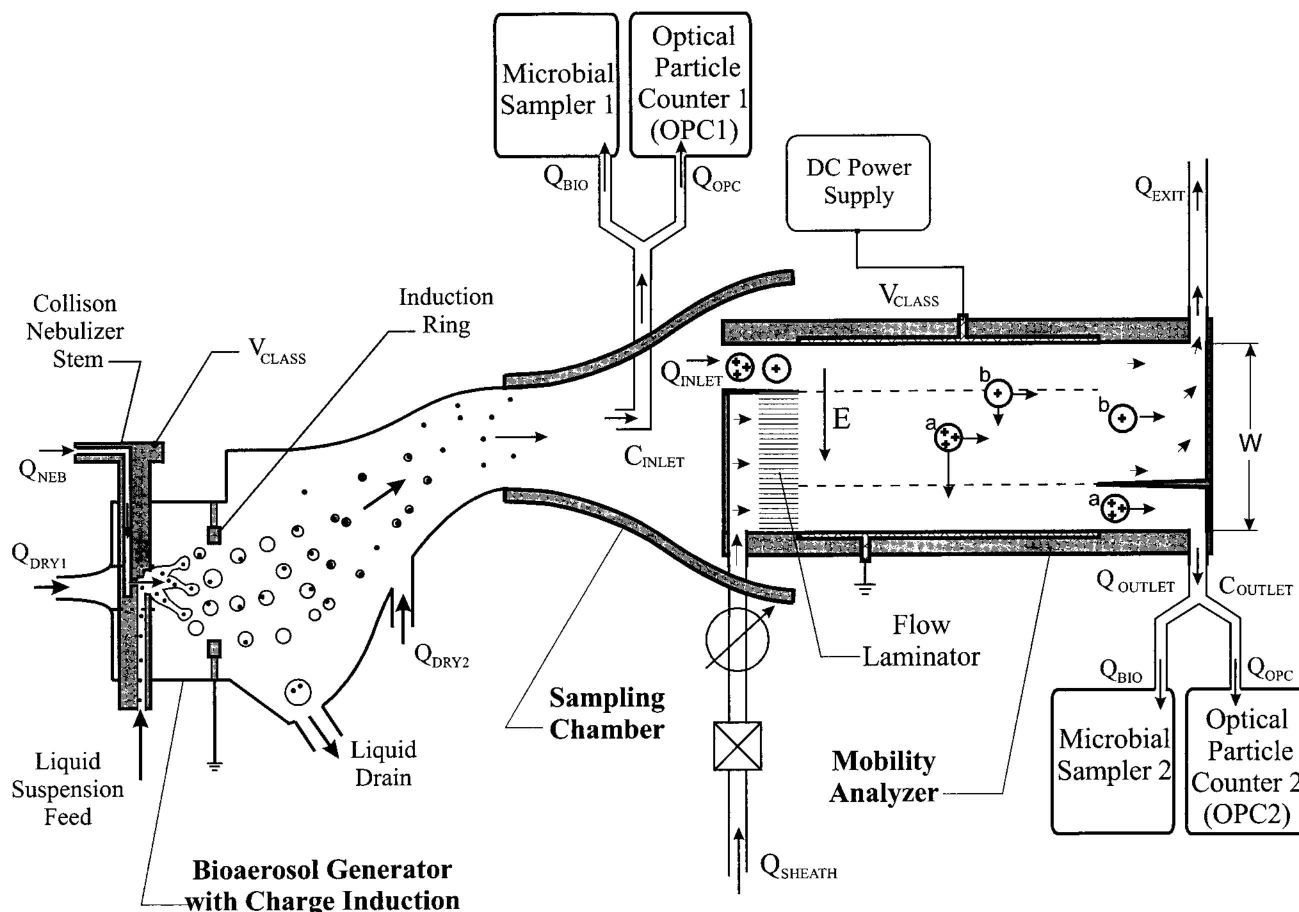

Figure 1. Experimental setup. The dry particles may carry multiple positive or negative charges.

Supply HP-6516A, Hewlett Packard, Inc., Rockaway, NJ).

The charged microorganisms then enter an open and horizontally oriented sampling chamber where their concentration and size distribution, C_{INLET}, is monitored by Optical Particle Counter 1 (OPC1; Model 1.108, Grimm Technologies, Inc., Douglasville, GA). This particle counter is operated at flow rate $Q_{OPC} = 1.2$ L/min and measures the aerosol particle concentrations in 16 size channels, ranging from 0.3 to 20 μm. Because we were concerned with particles of bacterial size, only the first eight channels from 0.3 to 3 μm were used. The viability of the airborne microorganisms was determined using Microbial Sampler 1 (BioSampler, SKC, Inc., Eighty Four, PA). This sampler collects bioaerosols into a liquid by the combined forces of impaction and centrifugation and has been shown to collect airborne microbial particles with minimal damage to sensitive microorganisms (Lin et al., 2000; Willeke et al., 1998). Microorganisms are then drawn into a specially designed electrical mobility analyzer, a top view of which is shown in Figure 1. They enter through a channel at the top left at a flow rate $Q_{INLET} = 6.7$ L/min, parallel to clean sheath air entering at $Q_{SHEATH} = 20.1$ L/min. When a voltage potential, V_{CLASS}, is applied across this parallel plate device, an electrical field, E, is created. This electrical field (positive in this example) deflects the charged particles from their linear motion toward the grounded plate (lower plate in Fig. 1). How much a charged particle will be deflected from its linear motion depends on the strength of the applied electric field, E, the diameter of the particle, and the magnitude of the electric charge on the particle. At a given V_{CLASS}, microorganisms of a specific charge level (marked **a** in Fig. 1) are extracted through the channel at the bottom right at flow rate, $Q_{OUTLET} = 6.7$ L/min, while the others (marked **b**) exit with the larger exit flow, $Q_{EXIT} = 20.1$ L/min. The extracted microorganisms are collected by Microbial Sampler 2, operated at $Q_{BIO} = 5.5$ L/min. The concentration of the extracted microorganisms, C_{OUTLET}, is measured by Optical Particle Counter 2 (OPC2) (Model 1.108, Grimm). The feasibility of using a mobility analyzer in conjunction with an optical particles counter has been shown by Biermann and Bergman (1984) and Emets et al. (1991). This technique has also been used in one of our previous studies (Mainelis et al., 2002). The latter research has shown that vegetative cells of *P. fluorescens* entering and exiting the analyzer have a median diameter of 0.73 μm, as measured by OPC1 and OPC2. Because the applied V_{CLASS} and the representative par-

ticle diameter are known, we can determine the number of elementary electric charges, n, carried by the extracted particles. For a given V_{CLASS}, Microbial Sampler 2 collects only the microorganisms carrying a certain electric charge, n. Because the electrical mobility analyzer has limited resolution, some microorganisms carrying electric charges lower or higher than n are also extracted. However, only particles carrying the selected electric charge value, n, are extracted with an efficiency of 100%, while particles carrying lower or higher charges are extracted with <100% efficiency. Thus, the median electric charge carried by the extracted particles is n when a certain V_{CLASS} is applied (Mainelis et al., 2002).

To minimize losses of charged particles in the system, all sampling lines were made of metal and all devices measuring and collecting charged particles were positioned as closely as possible to the particle source. All airflow rates in the system were monitored with flow meters calibrated with a Buck calibrator (A. P. Buck, Inc., Orlando, FL). The entire test system was placed in a Class II, Type B2 biological safety cabinet (SterilchemGARD; Baker Co., Sanford, ME) so that the uncollected aerosol particles were properly removed. The temperature was kept at 22° to 26°C and the relative humidity of drying airflow at 30% to 50% during all experiments. These parameters were monitored by sensors (Models TRH-100-20FT and P300-5PSID, Pace Scientific, Inc., Charlotte, NC) and recorded by a Pocket Logger (Model XR440, Pace Scientific) connected to a personal computer.

Procedures for Determining Magnitude and Polarity of Electric Charges Carried by Microorganisms

Initially, we determined the entire range of electric charges (from high negative to high positive) carried by the microorganisms. This was done by measuring the magnitude and polarity of electric charges carried by the microbial cells immediately upon their dispersal ($V_{INDUCTION} = 0$ V) through the stem of the Collison nebulizer. Then, we imparted either negative charges ($V_{INDUCTION} = -3000$ V) or positive charges ($V_{INDUCTION} = +3000$ V) during the dispersal of microorganisms. For each charging condition, the classifier's voltage, V_{CLASS}, was increased in a stepwise manner from 0 to −4500 V for recording the concentrations of aerosols carrying a net negative charge, and from 0 to +4500 V for recording the concentrations of aerosols carrying a net positive charge. Two stable external power sources (DC Power Supply HP6516A, Hewlett Packard, and DC Power Supply RHR, Spelman, Bronx, NY) supplied the analyzer's classification voltages. For each V_{CLASS} value, the concentration of microbial cells entering the analyzer, C_{INLET}, and the concentration of microorganisms extracted by the mobility analyzer, C_{OUTLET}, were simultaneously measured with OPC1 and OPC2 for 30 seconds. By computing the ratio of C_{OUTLET} to C_{INLET} we determined the fraction of microorganisms carrying n elementary electric charges at a given V_{CLASS}. By performing the same calculation for all V_{CLASS} values we determined the entire electric charge distribution for microorganisms of 0.73 μm diameter at a specific charging condition (this is the mean size of the microorganisms used in our experiments; the majority of the bacteria, almost 95%, are counted in the three size channels between 0.5 and 1 μm). The overall electric charge distribution of the microorganisms was determined three times for each charging condition ($V_{INDUCTION} = 0, -3000$, and $+3000$ V). The average values and standard deviations of these measurements are presented in the Results section.

Collection of Microorganisms Carrying Specific Electric Charges

From each electric charge distribution, determined at different charging conditions, we selected several charge values for the viability experiments so that the entire spectrum of electric charges (from high negative to high positive) carried by the microorganisms in their airborne state would be represented. The selected electric charge values are indicated in the Results section. The microorganisms carrying a specific electric charge were collected by the Microbial Sampler 2 (BioSampler, SKC, Inc.). This sampler contained 30 mL of phosphate buffer and was operated for 30 min. The buffer was prepared by dissolving 13.6 g of K_2HPO_4 and 4 g of KH_2PO_4 in 1 L of deionized filtered water (5 Stage Milli-Q Plus System, Millipore Corp., Bedford, MA). At the beginning and end of microorganism dispersion in a selected charging field, a representative sample of all microorganisms entering the electric mobility classifier was collected by Microbial Sampler 1 (BioSampler, SKC). This sampler also contained 30 mL of phosphate buffer and was operated for 30 min. The flow rates through each of these samplers was $Q_{BIO} = 5.5$ L/min.

Test Microorganisms

Stock cultures of *Pseudomonas fluorescens* (*P. fluorescens*) (ATCC 13525) were obtained from the American Type Culture Collection (Rockville, MD). *P. fluorescens* is a rod-shaped bacterium with a physical diameter of 0.7 and 0.8 μm and a length of 2.0 and 3.0 μm (Palleroni, 1984). Dry spores of *Bacillus subtilis* var. *niger* (BG spores) were obtained from the U.S. Army Edgewood Laboratories (Edgewood Research, Development and Engineering Center, Aberdeen Proving Grounds, MD). BG spores are often used by the Armed Forces in testing new biosensors for their ability to respond to particles of biological threat, such as airborne spores *Bacillus anthracis*, which causes anthrax. BG spores are rod-shaped,

approximately 0.7 to 0.8 μm in diameter and 1.5 to 1.8 μm in length (Johnson et al. 1994).

The BG spores were received in dry form and did not need additional cultivation. Prior to their aerosolization, a small amount of the spores was dissolved in sterile deionized water. This suspension was then kept at 55°C for 25 min to activate the spores. The *P. fluorescens* culture was grown in Trypticase Soy Broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) at 30°C for 18 h in a Gyrotory Water Bath Shaker (Model G76, New Brunswick Scientific, Inc., Edison, NJ). The *P. fluorescens* cells were harvested from their suspensions by centrifugation at 5050 × *g* for 7 min (Sorval RC-5B, Sorval Co., Newtown, CT). The resulting pellets were washed three times with sterile deionized water (5 Stage Milli-Q Plus). To obtain suspensions of desired bacterial density, the initial suspensions of both microorganisms were diluted with sterile deionized water. The concentrations of aerosolized *P. fluorescens* cells and BG spores ranged from 400 to 800 organisms/cm^3.

Determination of Viability and Injury of Test Microorganisms

The effect of electric charge on the viability of bacteria collected with Microbial Sampler 1 (all microorganisms) and Microbial Sampler 2 (microorganisms carrying *n* electric charges) was determined by measuring their relative recovery. The relative recovery of *P. fluorescens* cells and *B. subtilis* var. *niger* spores in each sample was determined as the ratio of the number of culturable bacteria, N_{TSA}, to the total number of bacteria, N_{TOT}, after this ratio was corrected by the initial culturability of the suspension, $F_{CULTURABLE}$:

$$\text{Relative recovery} = N_{TSA}/N_{TOT}F_{CULTURABLE} \qquad (1)$$

$F_{CULTURABLE}$ was determined prior to the aerosolization experiments as the ratio of the number of culturable bacteria in the suspension, N_{TSA}(suspension), to the total number of bacteria in the suspension, N_{TOT}(suspension):

$$F_{CULTURABLE} = N_{TSA}(\text{suspension})/N_{TOT}(\text{suspension}) \qquad (2)$$

The numbers of culturable bacteria in each air sample and in the suspension, N_{TSA} and N_{TSA}(suspension), respectively, were determined by taking aliquots from each air sample and suspension, diluting, and cultivating them on TSA agar (Becton Dickinson) in triplicate. After incubating *P. fluorescens* for 24 h at 30°C and BG spores for 24 h at 37°C, the formed colonies were counted and the obtained numbers constituted N_{TSA} and N_{TSA}(suspension), respectively. Only the counts from dilutions that had between 30 and 300 colonies per plate were used to calculate the average count of culturable bacteria, N_{TSA} and N_{TSA}(suspension), for use in Eqs. (1) and (2). The total numbers of bacteria in each air sample and in the suspension, N_{TOT} and N_{TOT}(suspension), respectively, were determined by staining the bacteria with acridine orange (AO) stain (Sigma Co.) and counting them under an epifluorescence microscope (Model Laborlux S; E. Leitz, Inc., available from Nuhsbaum, Inc., McHenry, IL). To standardize our procedures, when using Eq. (1), the number counts of the culturable and total microorganisms were converted to numbers per milliliter.

The metabolic and structural injuries of *P. fluorescens* bacteria, collected with Microbial Samplers 1 and 2, were also determined to evaluate more closely the physiological responses of the sensitive bacteria when aerosolized to an electrically charged state carrying *n* elementary electric charges. Structurally injured bacteria have been defined (Ray and Speck, 1973) as the survivors that are able to multiply and form colonies on a nonselective complete agar medium, but not on a selective medium containing some inhibiting agents. Ray and Speck (1973) defined metabolically injured bacteria as the survivors that are able to multiply and form colonies on a complete agar, but not on an agar that contains inorganic salts (such as a minimal salts medium). The extent of bacterial injury in each sample was determined by taking an aliquot from the sample, diluting, and plating it on two types of selective media in triplicate. Minimal salts glucose agar (MA) was used for determining the level of metabolic injury in the bacteria; MacConkey's agar (MC) was chosen for determining the level of structural injury (Stewart et al., 1995). After incubation for 24 h at 30°C, the number of colonies formed on minimal agar, N_{MA}, and the number of colonies formed on MacConkey's agar, N_{MC}, were obtained. The level of bacterial injury in each sample was determined by the difference between the count on the complete medium, N_{TSA}, and that on minimal or MacConkey's agar (N_{MA} and N_{MC}, respectively):

$$\text{Metabolic injury} = (N_{TSA} - N_{MA})/N_{TSA} \qquad (3)$$

and:

$$\text{Structural injury} = (N_{TSA} - N_{MC})/N_{TSA} \qquad (4)$$

Also, prior to every aerosolization experiment, we determined the average metabolic and structural injuries of bacterial cells in the suspension, designated as *metabolic injury* (*suspension*) and *structural injury* (*suspension*), respectively, using the described procedures. For presenting the levels of bacterial injury, we calculated the relative injury factors:

$$\text{Relative metabolic injury factor} = \text{metabolic injury}/\text{metabolic injury (suspension)} \qquad (5)$$

$$\text{Relative structural injury factor} = \text{structural injury} / \text{structural injury (suspension)} \quad (6)$$

The relative bacterial injury factors indicate the increase in injury due to the combined effect of aerosolization, electrical charging, and collection of bacteria.

Data Analysis

Data analysis was performed using analysis of variance (ANOVA) available as an add-in to Microsoft EXCEL 2000. $P < 0.05$ was considered statistically significant.

RESULTS

In the first set of experiments, we determined the electric charge distributions on *Pseudomonas fluorescens* bacteria when different induction voltages (−3000, 0, and +3000 V) were applied during aerosolization (Fig. 2). When *P. fluorescens* bacteria are aerosolized with no induction voltage applied (i.e., no additional electric charges are imposed) these bacteria are already highly charged (i.e., individual cell populations carry thousands of positive or negative electric charges), as seen in Figure 2B. Approximate integration of the areas under the curve indicates that the net electric charge on these bacteria is negative: about −4000 relative units. When the *P. fluorescens* bacteria are dispersed in a highly negative induction field, the net charge on the bacteria becomes more negative (close to −7000 units) and some of the bacteria carry up to 13,000 negative elementary electric charges, as shown in Figure 2A. When the bacteria are aerosolized in a highly positive induction field, the net charge on the bacteria becomes positive (about +1700 units), as shown in Figure 2C. In this case, the number of highly positively charged bacteria increases more than tenfold and we see individual bacteria carrying more than 10,000 positive elementary charges. For all three charging conditions, the lowest electric charge measured was 18 elementary charges, which is the limit of this experimental setup (Mainelis et al., 2002). Therefore, in Figure 2, the x-axis, "Number of elementary charges," has a gap from −16 to +16 elementary charge units. For all three charging conditions, a dip in the curves could be observed for n values lower than ±100 charge units. This may be due to the fact that the natural charge contained in cell membranes is usually higher than $n = \pm 100$ elementary charge units.

Based on the three electric charge distributions it appears that *P. fluorescens* cells can carry a very broad range of electric charges, ranging from >10,000 negative to >10,000 positive. However, to investigate how the viability of *P. fluorescens* cells depends on the magnitude of electric charges on the cells no single charging condition could be used alone. We selected the following electric charges, n, which cover most of the range of electric charges on the cells: −9200 and −30 elementary charges, when an induction voltage of −3000 V is applied; −4100, −830, −30, +30, and +2800 elementary charges, when no induction voltage is applied; and +30 and +7500 elementary charges, when an induction voltage of +3000 V is applied. Because the electrical mobility analyzer has limited resolution (Mainelis et al., 2002) some cells carrying electric charges lower and higher than n are also extracted. The extracted electric charge ranges are shown as triangles in Figure 2, because only cells carrying an electric charge n are extracted with 100% efficiency, whereas particles carrying lower or higher charges are extracted with <100; efficiency. Each triangle peak's value corresponds to a selected electric charge value, n.

Prior to our experiments investigating how the viability of airborne microorganisms depends on the magnitude of electric charge we determined the initial

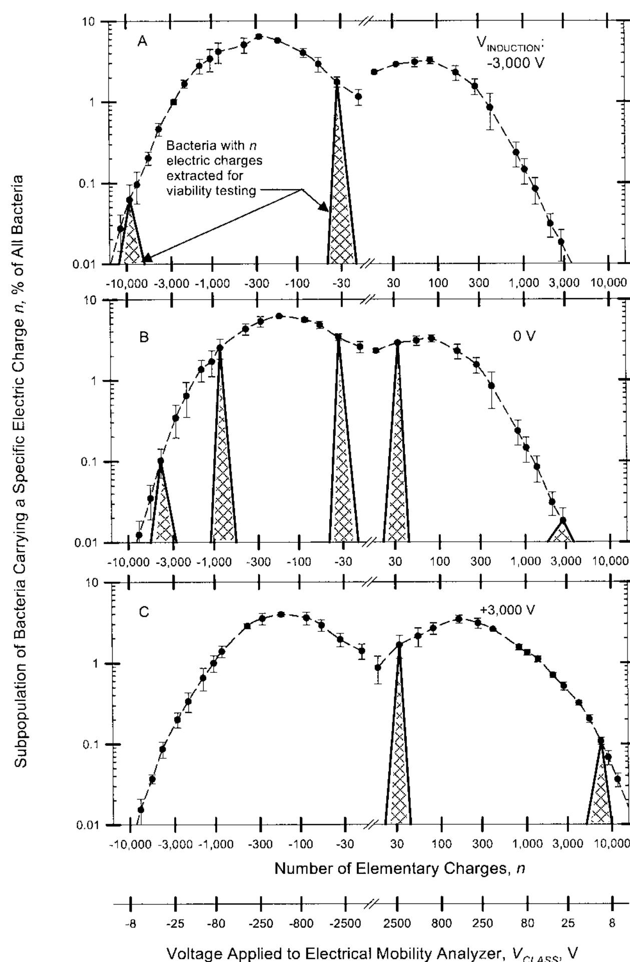

Figure 2. Electric charge distributions on *P. fluorescens* bacteria (0.65 to 0.8 μm) when different induction voltages are applied during aerosolization: (A) −3000 V; (B) 0 V; and (C) +3000 V. Error bars indicate the standard deviation from the means of three repeats.

culturability, $F_{CULTURABLE}$, of *P. fluorescens* in the liquid suspension of our aerosol generator and found it to be 45 ± 13%. This result is similar to 55 ± 18% reported by Terzieva et al. (1996). Other studies (Juozaitis et al., 1994; Thompson et al., 1994) have also reported <100% initial viability of *P. fluorescens*.

The relative recovery of airborne *P. fluorescens* cells carrying different amounts of electric charge was found to depend on the amount of electric charge, as shown in Figure 3. The data indicate that relative recovery of *P. fluorescens* carrying 30 negative charges is >60%, when an induction voltage of −3000 V is applied; this relative recovery decreases to about 20% for cells carrying a very large number (9200) of negative elementary charges. When no induction voltage is applied, the relative recovery of *P. fluorescens* cells carrying from 4100 negative to 30 positive elementary charges ranges from 40% to 60%, whereas the relative recovery of bacteria carrying 2800 positive elementary electric charges is <1.5%. When these cells are aerosolized with a positive induction voltage of 3000 V applied, their relative recovery is about 65% when they carry 30 positive charges. In contrast, the relative recovery of bacteria carrying a highly positive charge of 7500 is <1% for the same charging conditions. When the relative recovery was analyzed over the entire charge spectrum, it was found that the magnitude of electrical charge significantly affects the relative recovery of *P. fluorescens* bacteria ($P < 0.01$). The effect of charging condition on the relative recovery of bacterial cells was found not to be statistically significant ($P = 0.91$).

The relative recovery of cells carrying from 4100 negative charges to 30 positive charges was not different from the average relative recovery of cells collected with Microbial Sampler 1 (average relative recovery of all bacteria) ($P = 0.74$). The relative recovery of *P. fluorescens* carrying a high negative charge (−9200) was considerably lower than the average relative recovery of airborne cells in the sampling chamber ($P < 0.01$). The relative recovery of cells carrying very high positive elementary charges of 2800 and 7500 was also significantly lower than the average relative recovery of bacterial cells in the sampling chamber ($P < 1 \cdot 10^{-6}$).

In these experiments, the aerosolization of bacteria at each charging condition ($V_{INDUCTION} = -3000$ V, 0 V, + 3000 V) lasted about 3 h. During the experiments, the average viability of all aerosolized *P. fluorescens* bacteria varied from 37% to 48%, as measured at the beginning of aerosolization ($t = 0$ h), and from 44% to 51%, as measured at the end of aerosolization ($t = 3$ h) (see

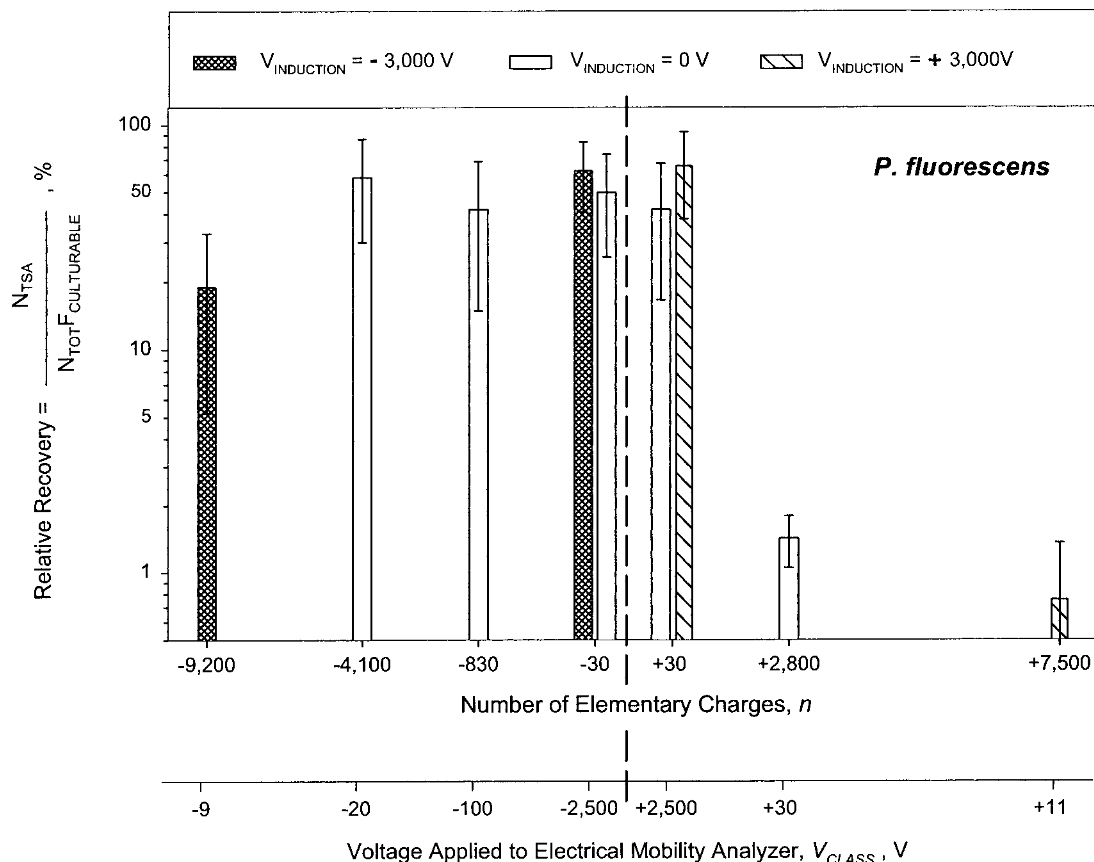

Figure 3. Relative recovery of *P. fluorescens* bacteria carrying different number of elementary charges, *n*, when different charging voltages are applied during aerosolization. Error bars indicate the standard deviation from the means of three repeats.

Table I. Relative recovery of *P. fluorescens* bacterial determined in the sampling chamber at the beginning and end of aerosolization when different induction voltages ($V_{INDUCTION}$) are applied during aerosolization.

	$\frac{N_{TSA}}{N_{TOT}F_{CULTURABLE}}$ (%)		
	$V_{INDUCTION}$ (V)		
Collection conditions	−3000	0	+3000
Beginning of aerosolization (0 h)	45 ± 8	48 ± 16	37 ± 17
End of aerosolization (3 h)	51 ± 30	44 ± 14	46 ± 16

Table I). Analysis of the data by analysis of variance (ANOVA) showed that the average viability of all *P. fluorescens* bacteria (collected from the sampling chamber by Microbial Sampler 1) was not affected by the charging voltage applied during the process of aerosolization dispersal ($P = 0.70$) or the aerosolization time ($P = 0.82$).

During these experiments, we also monitored the metabolic and structural injuries of *P. fluorescens* bacteria in the sampling chamber (bacteria not differentiated by the amount of carried electric charge) and injuries of the cells exiting the mobility analyzer (cells carrying a specific electric charge). The relative metabolic and structural injury factors averaged for all of *P. fluorescens* cells present in the sampling chamber, when different induction voltages are applied during the aerosolization, are presented in Figure 4. A relative injury factor of 1 indicates that there was no additional injury when compared with that in the bacterial suspension, and a relative injury factor >1 indicates that additional injury has occurred. The data show that, for each induction voltage, the increases in both metabolic and structural injuries at the beginning of the aerosolization (0 h) were very similar to those at the end of the aerosolization (3 h). The metabolic and structural injuries of bacteria aerosolized with either 0V or −3000V induction voltages applied increased by a factor of 1.3 to 1.7 relative to their values in liquid suspension prior to aerosolization (Fig. 4A and B, respectively). The average metabolic and structural injuries of *P. fluorescens* bacteria in liquid suspension were 41% and 31%, respectively. These values represent injuries averaged for all prepared suspensions. When an induction voltage of +3000 V was applied, the metabolic and structural injuries increased by a factor of 1.9 to 2.1, as seen in Figure 4C. However, as analyzed by ANOVA, the observed differences in relative injuries for different charg-

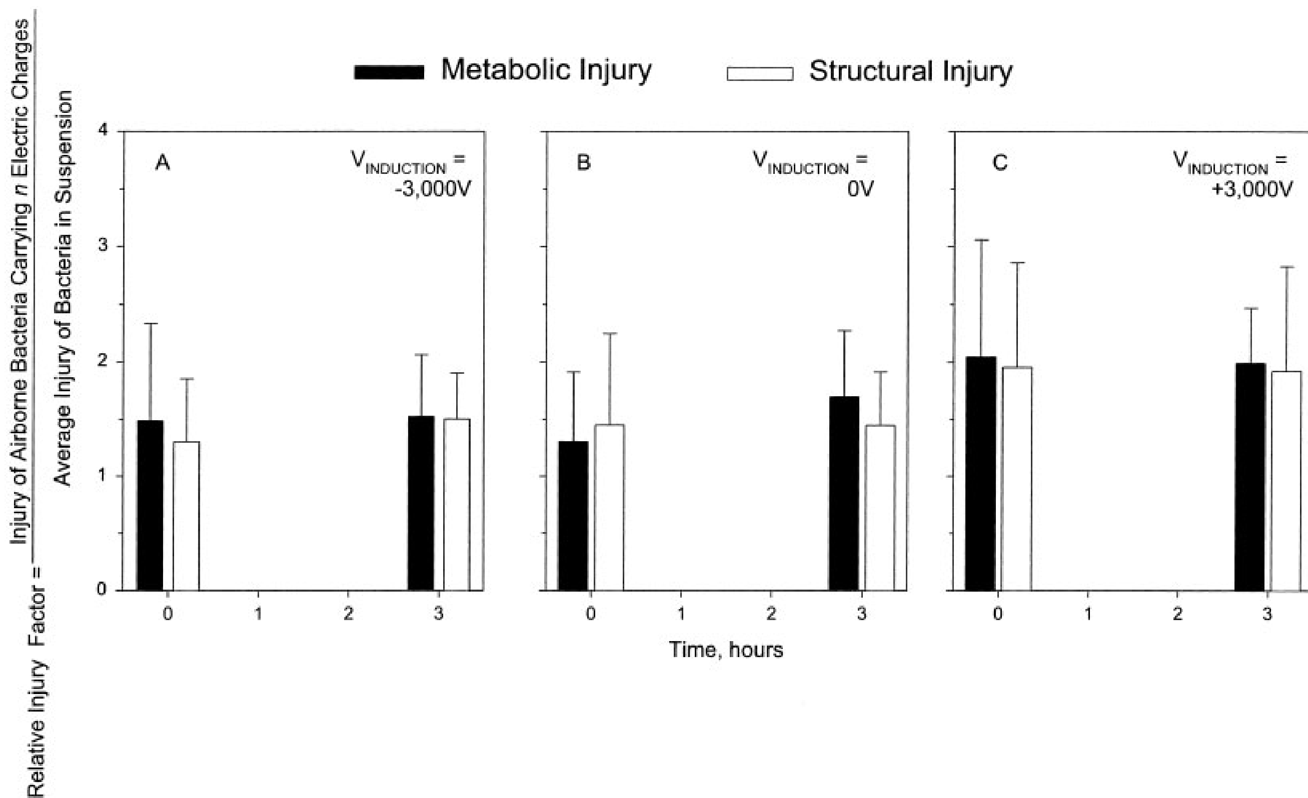

Figure 4. Relative metabolic and structural injury factors of *P. fluorescens* bacteria collected at the beginning and at the end of experiments when different charging voltages are applied during aerosolization: (A) −3000 V; (B) 0 V; and (C) +3000 V. Error bars indicate the standard deviation from the means of three repeats. A relative injury factor of 1 indicates that there was no additional injury when compared with that in the bacterial suspension, and a relative injury factor of >1 indicates that additional injury has occurred.

ing conditions were not statistically significant ($P = 0.25$ and 0.28 for metabolic and structural injury factors, respectively).

Figure 5 shows the relative metabolic and structural injury factors of *P. fluorescens* cells carrying different amount of electric charges, when different charging voltages are applied during microbial aerosolization. For all charging conditions, the relative metabolic injury factor was similar to that of structural injury: the relative metabolic injury of these bacteria increased by a factor of 1.5 to 2.6, whereas the extent of their structural injury increased by a factor of 1.3 to 2.6 times, when compared with the basal level of injuries of *P. fluorescens* cells prior to their aerosolization. The larger increases in metabolic injury, 2.4 and 2.6 times, was observed for bacterial cells carrying 2800 and 7500 positive electric charges, respectively. The largest relative structural injury factor, 2.6, was found for bacteria carrying 2800 positive elementary charges. However, as analyzed by ANOVA, the increases in metabolic and structural injuries were not significantly affected by the amount of electric charge carried by the bacterial cells ($P = 0.98$ and $P = 0.57$, respectively).

In our next set of experiments, the effect of the magnitude and polarity of electric charges on the viability of *B. subtilis* var. *niger* (BG) spores was investigated (Fig. 6). The relative recovery of airborne BG spores was determined for spores carrying electric charges at levels similar to those used during the viability investigations of *P. fluorescens* bacterial cells: −9200 elementary electric charges, when an induction voltage of −3000 V was applied; −4100, −830, −30, +30, and +2800 elementary electric charges, when no induction voltage was applied; and +7500 elementary electric charges, when an induction voltage of +3000 V was applied. Because the relative recovery of sensitive *P. fluorescens* bacteria carrying 30 positive or negative electric charges did not depend on whether these bacteria were additionally charged by induction charging, these conditions were not included when investigating the relative recovery of BG spores in view of their stress-resistant nature. The relative re-

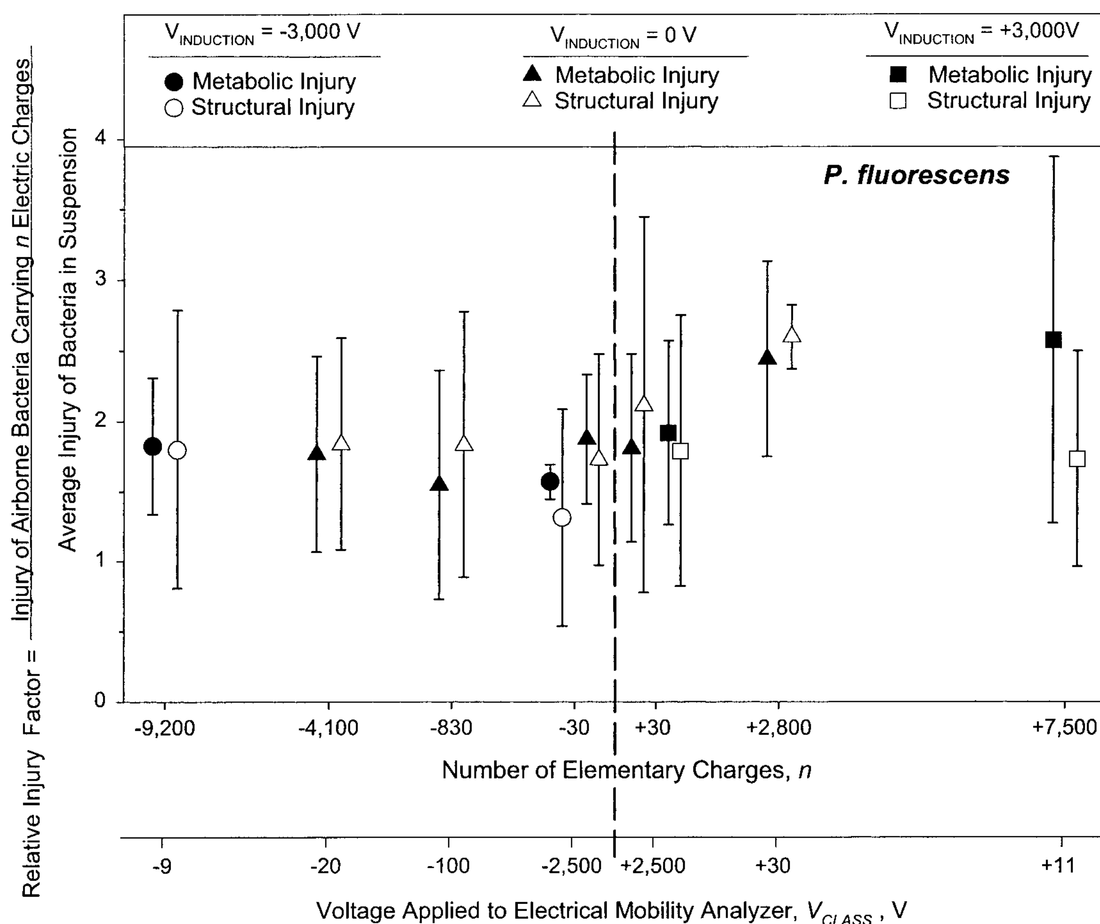

Figure 5. Relative metabolic and structural injury factors of *P. fluorescens* bacteria carrying different amounts of elementary electric charges, n, when different charging voltages are applied during aerosolization. Error bars indicate the standard deviation from the means of three repeats. A relative injury factor of 1 indicates that there was no additional injury when compared with that in the bacterial suspension, and a relative injury factor of >1 indicates that additional injury has occurred.

covery of BG spores varied from 44% to 81% over the investigated electric charge spectrum and was not affected by the amount of electric charge ($P = 0.76$) (see Fig. 6). The spores entering the sampling chamber—that is, spores not yet differentiated by the amount of electric charge on them—had an average viability of 71%. Analysis by ANOVA showed that this value was not different from the viability of spores differentiated by the amount of electric charge on them ($P = 0.83$).

DISCUSSION

Classification Voltage

The classification voltage, V_{CLASS}, applied to the electric mobility analyzer, determines the electric charge level on the extracted particles. The comparison of n-scale versus V_{CLASS} scale (bottom of Fig. 3) shows that microorganisms carrying high electric charges are extracted by applying a low voltage to the mobility analyzer, whereas the microorganisms carrying low charges are extracted by applying a high voltage; for example, $n = +30$ corresponds to $V_{CLASS} = +2500$ V, whereas $n = +7500$ corresponds to $V_{CLASS} = +11$ V. The relative recovery of sensitive *P. fluorescens* bacterial cells carrying +30 electric charges was not statistically different from the average relative recovery of all cells in the sampling chamber. Because cells carrying +30 charges are extracted when a classification voltage of +2500 V is applied to the mobility analyzer, it could be concluded that this voltage did not affect the viability of airborne bacteria. The voltage applied to the mobility analyzer creates an electric field, E_{CLASS}:

$$E_{CLASS} = V_{CLASS}/W \tag{7}$$

where W is the width of the mobility analyzer, shown in Figure 1. In our classifier, $W = 1.2$ cm. For a V_{CLASS} value of +2500 V, the E_{CLASS} would be approximately 2000 V/cm. Thus, our data show that electric fields of up to about 2000 V/cm can be used to electrostatically remove sensitive biological particles from the air stream without affecting their viability.

Possible Causes of Inactivation of Bacteria by High Electric Charges

The obtained data show that a high electric charge inactivates *P. fluorescens* cells, whereas an electric charge

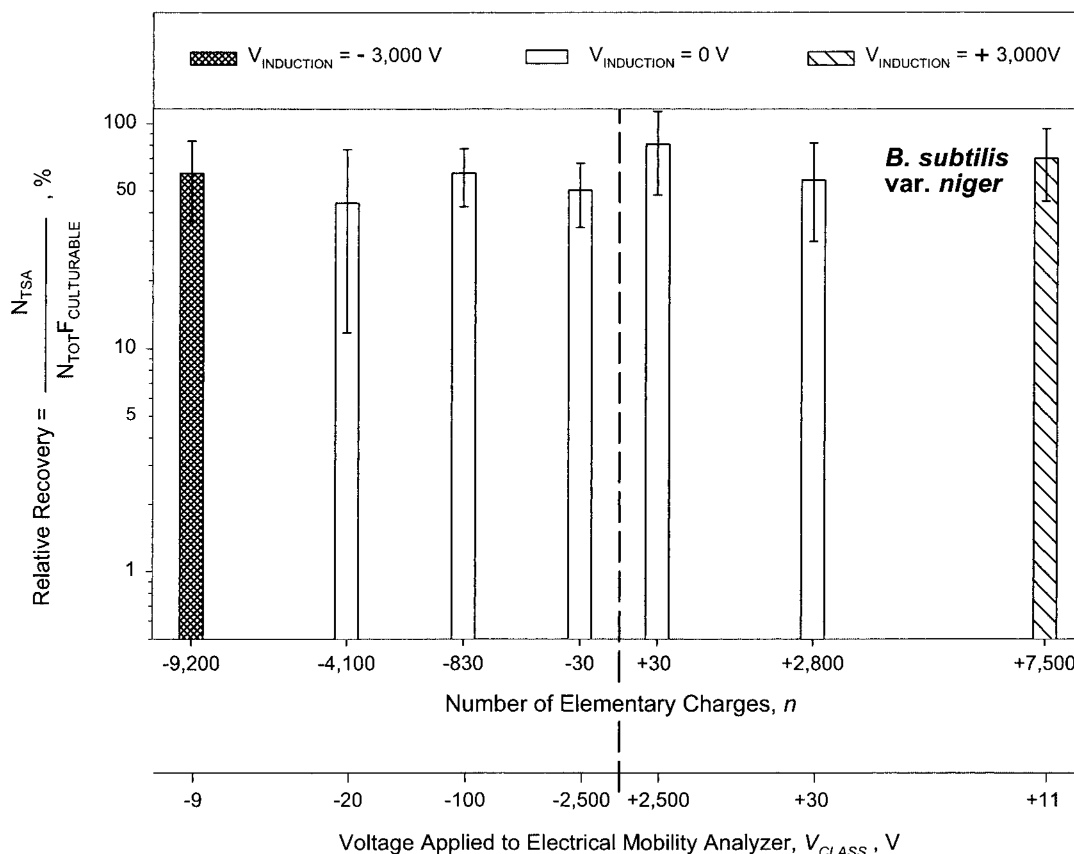

Figure 6. Relative recovery of *B. subtilis* var. *niger* bacterial spores carrying a different number of elementary charges, n, when different charging voltages are applied during aerosolization. Error bars indicate the standard deviation from the means of three repeats.

of the same magnitude and polarity does not inactivate BG spores. It appears that this difference is due to the difference in the nature of these microorganisms: that is, vegetative cells vs. microbial spores. Microbial spores are relatively resistant to extreme environments such as high temperature, high osmotic pressure, high and low pHs, toxic compounds, and mechanical shocks, compared with vegetative cells; furthermore, although they contain intracellular proteins (enzymes), nucleic acids, membranes, and structures of microbial spores similar to those of vegetative cells (Yonemoto et al., 1992), their core membrane lies inside a thick cortex and coat layers. These outermost layers protect bacterial spores from potentially damaging agents (Hamilton and Sale, 1967).

The difference between vegetative cells and microbial spores is clearly demonstrated when these microorganisms are suspended in liquids and exposed to high electric fields. Endospores of *Bacillus cereus* suspended in UHT-milk (1.5% fat) were shown to withstand electric fields as high as 22.4 kV/cm, whereas cells of *P. fluorescens* suspended in the same liquid were inactivated by a lower field of 11.5 kV/cm (Grahl and Märkl, 1995). Yonemoto et al. (1992) reported that spores of *B. subtilis* var. *niger* were highly resistant to treatment by electric field pulses, and little decrease in their viability was observed, whereas vegetative cells of *B. subtilis* var. *niger* decreased sharply in viability when exposed to the same electric field pulses.

When a bacterium is still suspended in liquid, its membrane's electric potential is determined by the transmembrane potential, V_M, between the cytoplasm and extracellular fluid, and two surface potentials at the external, V_{OE}, and internal, V_{OI}, interfaces of the membrane. At equilibrium, the external surface potential, V_{OE}, is counterbalanced by ions of opposite charge (counter-ions) (Krekeler et al., 1989). Because the membrane of a bacterial cell has a certain electrical capacity (Glaser, 1992), an applied external electric field charges this "bacterial capacitor," thus generating transmembrane potential, V_G. This potential is superimposed upon the resting transmembrane potential, V_M. Combined transmembrane potentials, $V_G + V_M$, of >1 V are believed to permeabilize the cell membrane by pore formation (Zimmermann et al., 1980, 1988), which leads to inactivation of the microorganism. Membrane breakdown occurs in nanoseconds after the breakdown voltage has been reached (Zimmermann, 1996).

In an effort to explain our experimental data quantitatively, we have made the following estimates. For the microorganisms carrying hundreds or thousands of elementary electric charges, we assumed that the "bacterial capacitor" is at least partially charged. The voltage potential between an electric capacitor's plates, V, is:

$$V = q/C \tag{8}$$

where q is electrical charge and C represents the electrical capacitance. According to Tonoyan et al. (1989), bacteria, on average, have a capacitance of 1 $\mu F/cm^2$. For a *P. fluorescens* cell with an average diameter 0.73 μm this corresponds to an electric capacity of $1.7 \cdot 10^{-14}$ F. If, for example, the microorganism carries 3000 elementary electric charges, Eq. (9) indicates that the transmembrane potential generated by these charges is approximately 30 mV, which is considerably less than the $V_G + V_M = 1$ V required to induce membrane permeabilization of cells suspended in water (Zimmermann et al., 1980, 1988).

However, when microorganisms are dispersed from liquid into air, their extracellular environment changes from liquid to air, which has different pH and conductivity properties. Fragments of the bacterial surface and some counter-ions may be removed by the shear forces of aerosolization, as indicated by conductivity changes in the bacterial suspension and the dispersion process itself may also impose electric charges onto the microorganisms (Mainelis et al., 2001). Some of the aerosolized *P. fluorescens* populations were measured to have a net positive electric charge, resulting in a positive surface potential, which is opposite to the negative potential of these microorganisms suspended in a liquid (Yamane et al., 1970). Thus, microorganisms in the airborne state undergo significant changes in their surface potential. Bacterial cells depend on their membrane potentials for their basic metabolic activity (Cevc, 1990), and alterations in the membrane potential have been shown to significantly affect ion transporters/channels and metabolically essential proteins, such as ATPase (Bond and Russel, 2000; Elinder and Arhem, 1999). Therefore, it is likely that the electric charging affects the membrane potential of sensitive microorganisms so significantly that their cells, especially those that are already injured, become nonviable.

When electric charges are imposed onto microorganisms either by the aerosolization process or by induction charging, these charges create local electric fields. It has been shown that low-intensity electric fields (1.5 to 20 V/cm) can change bacterial properties, such as their inherent resistance to biocides and antibiotics (Costerton et al., 1994). The hydrophobic core of the membrane phospholipid bilayer has a very low dielectric constant. Therefore, very large electric fields may be induced around localized electric charges. This, in turn, may influence the functional state of the membrane proteins (Glaser, 1996). Changes in conductivity of the cell membrane may alter the sensitivity of microorganisms to environmental and processing factors (Vega-Mercado et al., 1996).

The difference between *P. fluorescens* cells (Gram-negative) and *B. subtilis* var. *niger* (Gram-positive) in

their response to high positive electric charges may also have been caused by the chemical differences of their cell wall structure. The cell walls of Gram-negative bacteria have a greater lipid content, whereas Gram-positive bacteria have more peptidoglycans in their cell walls. This may differently affect the charge-related orientation of metabolically linked proteins and other cell membrane components. A combination of the aforementioned factors seems to affect the viability of *P. fluorescens* cells carrying high electric charges. Further research with bacterial species having different cell wall components is needed to better understand the response of bacteria to electric charges.

CONCLUSIONS

Bacteria dispersed from a liquid by means of pneumatic nebulization have a wide and bipolar electric charge distribution. We conclude that one of the factors affecting the relative recovery of aerosolized bacteria is the electric charge imposed on them by the aerosolization process. Thus, in applications wherein the recovery of airborne bacteria is important, an aerosolization method should be chosen so that it imposes the least amount of high positive charges onto sensitive bacteria. Our experiments have also shown that the relative recovery of sensitive *P. fluorescens* vegetative cells depends significantly on the magnitude and polarity of their electric charges, whereas the relative recovery of *B. subtilis* spores was not affected by the amount of electric charges. These findings are useful for the development of new bioaerosol sampling methods based on electrostatic principles. They also facilitate applications of electric charging for environmental control purposes involving sterilization of bacterial cells by imposing high electric charges on them.

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