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Electrical charges on airborne microorganisms

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Abstract

We have investigated the parameters affecting the magnitude and polarity of the electric charges carried by biological particles in the airborne state. A recently developed experimental setup through which we analyzed the electric charges imposed on airborne particles by a means of induction charging (Mainelis et al. (Aerosol Sci. Technol. 2001, submitted for publication)) was utilized for this research. In this study, the microorganisms were aerosolized under controlled conditions and an electric mobility analyzer extracted particles of specific electric mobility. The extracted microorganisms were then analyzed by an optical particle size spectrometer. The amount of electric charge carried by airborne microorganisms was found to depend on the dispersion method and can be more than 10,000 elementary electric charges. This finding contrasts with the low electric charge levels carried by non-biological particles. Our data show that repeated pneumatic dispersion of sensitive bacteria affects their structural integrity, which, in turn, changes the magnitude of electric charges carried by these bacteria. We have concluded that the amount of electric charge carried by aerosolized bacteria may be used as an indicator of mechanical stress. It was also found that the electrical conductivity and the pH level of a bacterial suspension increase during aerosolization from a Collison nebulizer. Thus, these two parameters may be used as indicators of the mechanical stress, injury and loss in viability, endured by bacteria during aerosolization, i.e., measuring the electrical conductivity and pH level of bacterial suspensions may be a simple and convenient method for monitoring the "wear and tear" of the bacteria suspended in deionized water.

Keywords: Airborne microorganisms; Electrical charges; Aerosolization

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1. Introduction

Exposure to bioaerosols, especially to pathogenic or allergenic microorganisms, may cause a wide range of respiratory and other health disorders in occupational and general populations. Various illnesses and infections due to microbiological exposures have been found in indoor environments (Burge, 1990; Miller, 1992; Spengler et al., 1993; Koskinen et al., 1995; Morey et al., 1984), metal working fluid environments (Robertson, Weir, & Burge, 1988; Popendorf et al., 1996; Graves et al., 1997; Kriebel et al., 1997; Robins et al., 1997; Kennedy, Chang-Yeung, Teschke, & Karlen, 1999), textile manufacturing industries (Schachter, Maunder, & Beck, 1984), solid waste treatment facilities (Lembke & Kniseley, 1980; Rahkonen, Ettala, Laukkanen, & Salkinoja-Salonen, 1990), food processing industries (Cox & Wathes, 1995) and agricultural environments (Jacobs, 1994; Donham, 1990).

Exposure to airborne microorganisms is usually assessed by using air samplers designed for monitoring the microbial aerosols that are viable. When viable airborne particles are collected, their recovery, in general, is decreased by some inactivation or loss during or after sampling (Burge & Solomon, 1987; Eduard et al., 1990; Jensen, Todd, Davis, & Scarpino, 1992; Buttner & Stetzenbach, 1993; Buttner, Willeke, & Grinshpun, 1996). Several studies have been devoted to the development of aerosol samplers with the aim to not only collect airborne microorganisms efficiently, but also to preserve the biological integrity of each sample (Nevalainen, Pastuszka, Liebhaber, & Willeke, 1992; Thompson, Donnelly, Grinshpun, Juozaitis, & Willeke, 1994; Grinshpun et al., 1995; Lin et al., 2000). During the development of new bioaerosol samplers and during their subsequent evaluations and calibrations several characteristics of airborne microorganisms are measured, such as their sizes and concentrations. One parameter that is often overlooked is the electric charge on airborne particles. The effects associated with the electric charge are frequently ignored or invoked as a qualitative explanation for unexpected observations (Johnston, Vincent, & Jones, 1985). The electric charge can greatly influence particle deposition in sampling lines during their transport (Brockman, 1993) and plays an important role when aerosol particles are collected on filters (Shapiro, Laufer, & Gutfinger, 1983; Liu, Pui, Rubow, & Szymanski, 1985) or in small sampling cyclones (Briant & Moss, 1984). It has been shown that the amount of electric charge carried by inhaled particles can have a significant effect on their deposition in the lung (Melandri et al., 1977; Vincent, Johnston, Jones, & Johnston, 1981; Prodi & Mullaroni, 1985). Bailey (1997) has shown that the deposition in the lung of 1 μm particles carrying 100 elementary electric charges is about 10 times greater than that of electrically neutral particles.

Although the electric charge carried by non-biological airborne particles in occupational environments is an important parameter, limited data have been reported. Kosenko (1970) measured the electric charges on dusts in various workplaces, such as an iron-ore mine, a cement factory, a gypsum production plant, and a foundry, and found that the particles released in these environments may carry several dozen elementary electric charges. However, the author did not specify the size of examined particles. Walkenhorst (1971) reported that 1 μm particles released in an iron foundry may carry up to 100 elementary electric charges. To the best of our knowledge, no data have been reported for *airborne* microorganisms prior to this study.

Studies on water-borne microorganisms have indicated that microorganisms in a liquid may carry thousands of elementary charge units (Sherbet & Lakshmi, 1973). Thus, we expected that

microorganisms in the airborne state also carry high electric charges. Information on the electric charges carried by airborne biological particles is not only important for assessing the particle loss and collection characteristics in the human respiratory tract and in existing bioaerosol samplers, but also for the development of new bioaerosol samplers, particularly in samplers that utilize electrostatics for particle collection. The electrostatic technique has the potential for being a "gentle" collection method, since the particle velocity component perpendicular to the collection medium is about two to four orders of magnitude lower than that in bioaerosol impactors and impingers sampling at comparable flow rates (Mainelis et al., 1999). We have recently modified an electrostatic aerosol sampler initially designed for collecting non-biological particles (model 3100, TSI Inc., St. Paul, Minnesota) and evaluated its performance for collecting airborne bacteria. The data showed that hardy microorganisms, such as *Bacillus subtilis* var. *niger*, can be efficiently collected by electrostatic precipitation without loss of viability, while sensitive bacterial cells, such as *Pseudomonas fluorescens*, may be inactivated by the corona discharge, the charging mechanism in conventional electrostatic precipitators (Mainelis et al., 1999).

We hypothesized that the natural electric charge on airborne microorganisms may be sufficiently high so that microorganisms could be efficiently collected by electrostatic means without the use of a corona discharge. As a step toward confirming or rejecting this hypothesis, we have developed a new bioaerosol generator, in which the microorganisms can be electrically charged by induction, and a new electrical mobility analyzer for extracting microorganisms of specific charge ranges (Mainelis et al., 2000). In this study, which is part of broader investigation for development of an efficient and "gentle" bioaerosol sampler, we present the data obtained on the electric charges carried by biological particles aerosolized under controlled conditions. We also investigate some of the parameters that affect the electric charges on aerosolized microorganisms.

2. Characteristics of electric charges on water-borne microorganisms

Our assumption that microorganisms in the airborne state are electrically charged is based on information published on the electric charge characteristics of water-borne microorganisms. We will, therefore, first review the available data on electric charges carried by microorganisms suspended in a liquid.

2.1. Charge components of water-borne microorganisms

Electron microscopy of *Escherichia coli* and other Gram-negative bacterial cells has revealed the presence of three distinct layers in the cell wall. The innermost is a cell membrane which is surrounded by a rigid peptidoglycan layer. The outermost layer (outer membrane) contains lipopolysaccharides, phospholipids, and lipoproteins and is about 80 Å thick (Sherbet & Lakshmi, 1973). The electric charge on the surface of a bacterial cell is attributable to a large extent to the kind of ionizable groups present on the cell surface and to their spatial distribution. In Gram-negative bacteria the major contribution to their surface charge is made by ionizable amino (NH_2) and carboxyl (COOH) groups of proteins exposed at the cell surface (Gittens & James, 1963; Sherbet & Lakshmi, 1973). Such bacteria have been shown to have an overall net negative surface

charge. Acid lipopolysaccharides present in the outer membrane of Gram-negative bacteria also contribute to the negative charge level (Sutherland, 1977).

Sleytr (1978) has shown that the net negative charges on Gram-positive bacteria depend primarily on the presence of teichoic and teichuronic acids and acidic polypeptides (S-proteins). The coat of a Gram-positive endospore, such as *Bacillus subtilis* var. *niger*, is organized in three layers: an amorphous undercoat; a lamellar inner structure; and a striated electron-dense outer coat (Henriques & Moran, 2000). Some evidence suggests that the outermost coat layer may have properties that are distinct from the rest of the structure. According to Sherbet and Lakshmi (1973), ionizable groups of the polysaccharide–phospholipid–protein complex occur in the 60 Å-thick isoelectrical zone of the outermost layer. Krishna, Powell, and Borriello (1996) corroborated their conclusion by determining that most of the electric charges on *Clostridium difficile* reside in the cell wall.

The dissociated chemical groups, present in the bacterial surface, generate a surface potential which is counterbalanced by ions of the opposite charge (counter-ions) present in the liquid suspension. Thus, an electrical double layer is built up. According to the widely accepted Stern model (Krekeler, Ziehr, & Klein, 1989), the electrical double layer consists of two parts. The ions of the first part (the Stern layer) are attached strongly enough to overcome thermal and Van der Waals forces. In the second part, the ions are diffuse, and free movement is possible (James, 1979). The surface conductance of a bacterium depends on the ionic conductance of the counter-ions and the charge density in the double layer. Thus, the total surface charge density is usually divided into two parts, that of the diffuse layer and that of the fixed Stern layer (Gittens & James, 1963).

2.2. Charge measurements of water-borne microorganisms

One of the common methods to determine a microorganism's net electric charge is to obtain its electrophoretic mobility, which is a measure of the microorganism's movement in a solution when subjected to an externally applied electric field (Richmond & Fisher, 1973). The isoelectric point or the zeta potential or the electrophoretic mobility at pH 4 usually characterizes the surface charge of bacteria (Krekeler et al., 1989). It is possible to calculate the surface charge density of a microorganism from its zeta potential (Adamson, 1960; Brinton & Laufer, 1959). However, most researchers use only zeta mobility data, because of the uncertainties regarding several factors needed for the calculations. Experiments employing isoelectric equilibrium analysis have shown that the number of net negative charges on unmodified cells of *Escherichia coli* was tens of thousands per cell (Sherbet & Lakshmi, 1973). No fundamental differences between the isoelectric point of Gram-positive and Gram-negative microorganisms were observed (Krekeler et al., 1989).

The surface charge on biological particles can also be characterized by colloid or polyelectrolyte titration. This method is based on the fact that polyelectrolytes of opposite charge form complexes in a stoichiometric way and the endpoint of the complex formation can be determined colorimetrically by using indicators (Krekeler et al., 1989). Through utilizing this method (Noda, Katayama, & Kanemasa, 1984) determined the surface charge of *Micrococcus luteus*, and Van der Wal, Minor, Norde, Zehnder, and Lyklema (1997) found that the cell wall charge density in Gram-positive bacteria can be as high as 0.5–1.0 C/m^2. However, the accuracy of this method is usually not sufficient and, thus, colloid titration finds limited applications.

Another popular method for investigating the bacterial charge is by electrostatic interaction chromatography. This method is simpler than the zeta potential measurement and can be used to

examine interactions on parts of the cell surface, i.e., to determine localized charges (Hermansson, Kjelleberg, Korhonen, & Stenstrom, 1982). It has been noted, however, that electrophoretic measurements and interaction chromatography give different data about the surface charges on the bacteria (Hermansson et al., 1982; Jones, Adair, Mawhinney, & Gorman, 1996).

3. Materials and methods

3.1. Aerosol generators

In this study, we used three aerosol generators to disperse biological and non-biological particles: a standard Collison nebulizer (BGI Inc., Waltham, Massachusetts), a Collison nebulizer specially modified for our tests and a bubbling aerosol generator developed by Ulevicius et al. (1997). In a standard Collison nebulizer (Fig. 1A), high-pressure air, $Q_{\mathrm{NEB,A}} = 6$ l/min, is pushed through one, three or six nozzles from which it exits at high velocity. Each air jet exits at a static pressure which is less than that of the ambient air and pulls fluid up the tube from the liquid reservoir. This fluid is then broken up by the air jet into a dispersion of droplets of very wide size distribution (May, 1973). Most of the larger droplets are impacted onto the inner wall of the glass vessel and are thus recirculated into the liquid reservoir. Most of the liquid in a 20 ml fill recirculates every 6 s. We used a standard Collison nebulizer with three nozzles and operated it at a positive pressure of 20 psi (1.4×10^5 Pa). Gussman (1984) determined that the mass median diameter of droplets escaping a three-nozzle Collison nebulizer is 3 μm when operated at 20 psi.

In the modified Collison nebulizer, the suspension liquid is not recirculated: the particles are dispersed through a single orifice in the center stem of a Collison nebulizer and the nebulizer's housing, which normally acts as the impaction surface, is removed (Fig. 1B). The droplets are dispersed at a flow rate $Q_{\mathrm{NEB,B}} = 1.2$ l/min. Downstream of the nebulization point, airflow $Q_{\mathrm{DRY,B}} = 50$ l/min transports the dispersed droplets away and starts drying them. Since dispersion through the Collison's orifice produces droplets of a very wide size distribution, as large as 55 μm (May, 1973), and the impaction wall is removed, the generator is slightly inclined towards the ground so that the largest droplets settle to the bottom of the generator and are drained. Thus, the particles in the liquid suspension are either aerosolized or, once drained do not return to the suspension feed and thus cannot be subjected again to aerosolization. The modified Collison nebulizer was operated at a positive pressure of 20 psi (1.4×10^5 Pa). To minimize particle losses in the aerosol generator due to electrostatic attraction to non-conducting surfaces, all aerosol disperser parts were made of metal. When coupled with a charge induction section, this aerosol disperser can be used to manipulate charge levels on airborne biological and non-biological particles (Mainelis et al., 2001).

The bubbling aerosol generator (Ulevicius et al., 1997) aerosolizes microorganisms from a liquid by gentle bubble bursting (Fig. 1C). The external body of this device consists of a vessel with a cover. Bubbles are formed by passing dry air at a flow rate $Q_{\mathrm{BUB}} = 1.5$ l/min through a medium-porosity fritted disk which is immersed in the suspension. Two streams of drying air, tangentially injected at a flow rate $Q_{\mathrm{DRY,C}} = 15$ l/min, dry the generated droplets. The droplets rapidly shrink in size to their bacterial content or liquid residues and are carried out of the disperser by the inward swirling motion of the drying air. This aerosol generator was found to produce stable

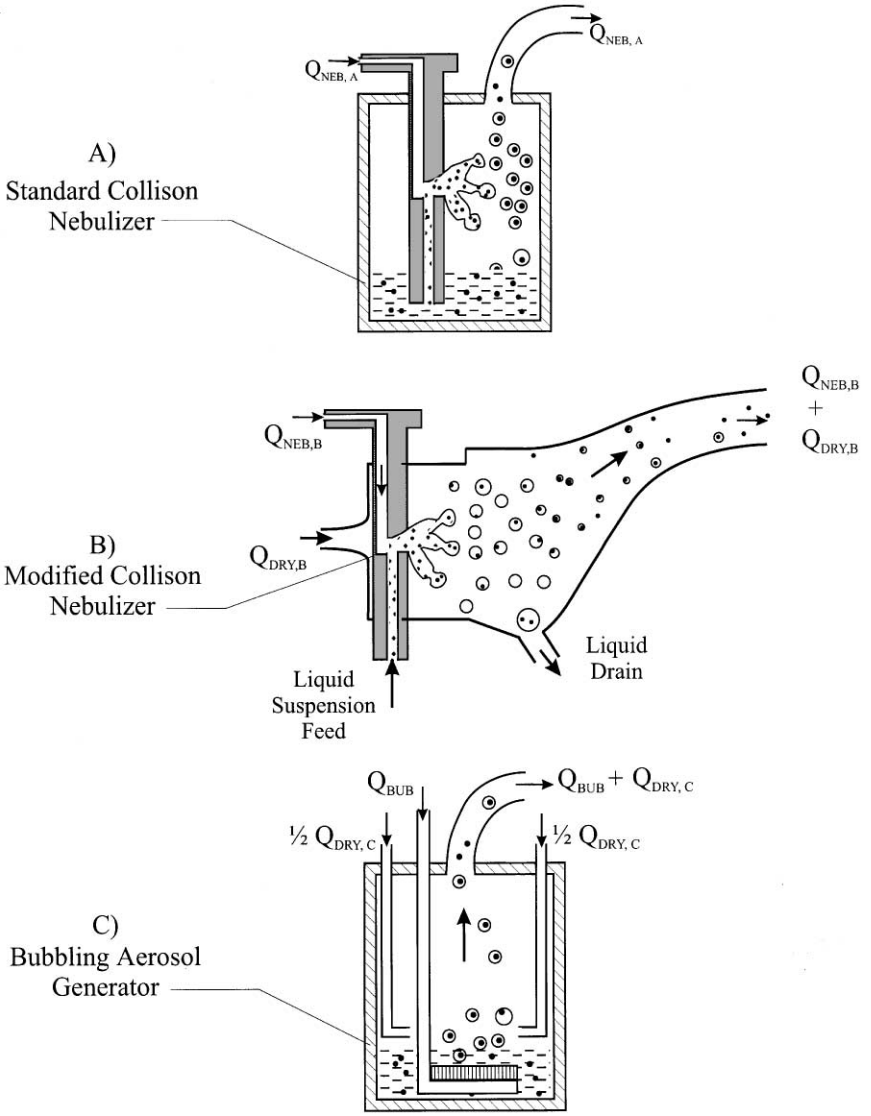

Fig. 1. Bioaerosol generators used in the study: (A) Standard Collison nebulizer; (B) modified Collison nebulizer; (C) bubbling aerosol generator.

concentrations of non-biological (Ulevicius et al., 1997) and biological (Reponen, Willeke, Ulevicius, Grinshpun, & Donnelly, 1997) particles.

3.2. Experimental setup

The experimental setup developed for this study is shown in Fig. 2. Biological and non-biological particles were aerosolized from a suspension by using one of the three aerosol generators described

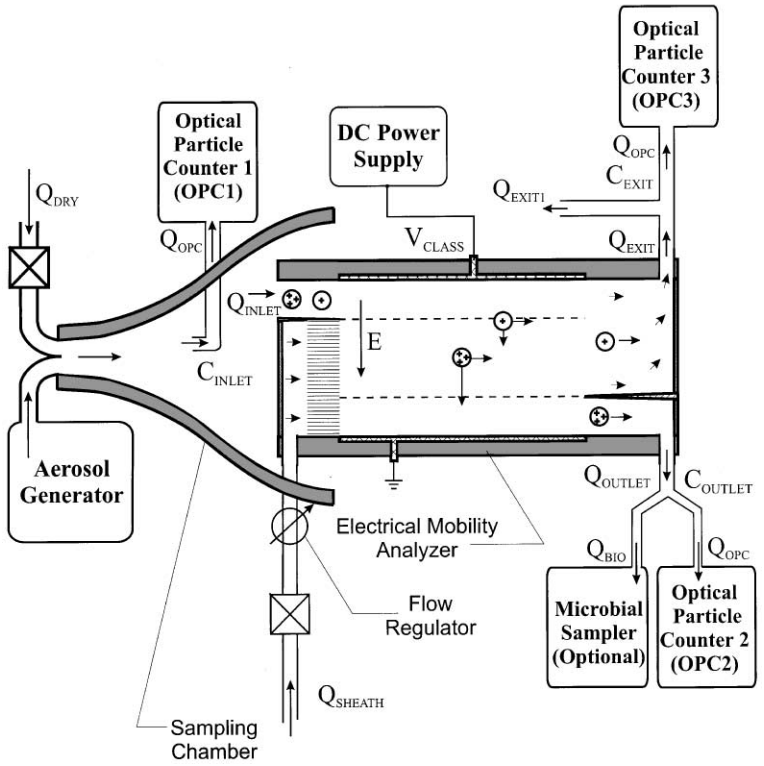

Fig. 2. Experimental setup.

above (generator used for a particular experiment is specified in the Results section). The droplets exiting from the aerosol generator were subjected to further drying by adding dry airflow, $Q_{DRY} = 30$ l/min. The dry particles and droplet residues then entered an open and horizontally oriented sampling chamber. The concentration and size distribution of particles in the sampling chamber, C_{INLET}, was monitored by an optical particle counter (OPC1) (model 1.108, Grimm Technologies Inc., Douglasville, Georgia). The measurement in this device is performed by quantifying the angular light scattering caused by the passage of particles through the laser light beam. The scattered light is collected at a 90° angle and then analyzed by the pulse height analyzer. In one of our experiments, airborne particles entering the sampling chamber were neutralized by inserting a 10 mCi 85Kr particle charge neutralizer between the sampling chamber and the aerosol generator (model 3012, TSI Inc., St. Paul, Minnesota; not shown in Fig. 2). The standard Collison nebulizer was used to disperse particles in that particular experiment.

An electrical mobility analyzer coupled with another optical particle counter (OPC2) (model 1.108, Grimm Technologies Inc.) sampled particles from the open sampling chamber and determined the magnitude and polarity of their electric charges. The feasibility of using a mobility analyzer in conjunction with an optical particle counter for measuring electric charges has been shown by Biermann and Bergman (1984) and Emets, Kascheev, and Poluektov (1991). A top view of this analyzer is schematically shown in Fig. 2. This device, described in detail by Mainelis et al.

(2001), has the following main features. The particles are drawn into the analyzer through a channel on the top left at flow rate $Q_{\mathrm{INLET}} = 6.7$ l/min, parallel to clean sheath air entering at $Q_{\mathrm{SHEATH}} = 20.1$ l/min. By applying a voltage potential, V_{CLASS}, across this parallel plate device, particles of specific electric mobility are extracted through the channel at the bottom right at flow rate $Q_{\mathrm{OUTLET}} = 6.7$ l/min. The concentration of these particles, C_{OUTLET}, is measured by the OPC2 operated at flow rate $Q_{\mathrm{OPC}} = 1.2$ l/min. To investigate the electrobiological properties of viable airborne microorganisms, we can optionally add a microbial sampler, as shown in Fig. 2. The remaining particles leave the electric mobility analyzer through the exit channel at the top right at flow rate $Q_{\mathrm{EXIT}} = 20.1$ l/min. In some of our experiments, the concentrations of particles leaving the analyzer through this exit channel (particles that are less deflected by the applied electrostatic field E), C_{EXIT}, were measured by a third optical particle counter (OPC3) (model 1.108, Grimm Technologies Inc.) that was also operated at $Q_{\mathrm{OPC}} = 1.2$ l/min. In this case, Q_{EXIT} was reduced by Q_{OPC} to $20.1 - 1.2 = 18.9$ l/min.

To minimize losses of charged particles in the system, all sampling lines were made of metal, and all particle analysis devices were positioned as close as possible to the particle source. All airflow rates in the system were monitored by flow meters calibrated with a Buck calibrator (A.P. Buck, Inc., Orlando, Florida). The entire test system was placed in a Class II, Type B2, biological safety cabinet (SterilchemGARD; Baker Company, Sanford, Maine) so that the uncollected aerosol particles were properly removed. The temperature was kept at 22–26°C and the relative humidity of the sheath and drying air flows at 30–50% during all experiments. These parameters were monitored by temperature and humidity sensors and recorded by a "Pocket Logger" connected to a personal computer.

3.3. Experimental procedures

At the start of each experiment, the system was operated without aerosolizing particles until zero particle background was achieved, as measured with the optical particle counters OPC1 and OPC2. In the next step, particle aerosolization was activated and the OPC2 readings were checked to ensure that no particles passed through the analyzer's outlet when no classification voltage was applied. Next, the analyzer's voltage, V_{CLASS}, was increased in a step-wise manner from 0 to -4500 V, for measuring particles carrying a net negative charge; and from 0 to $+4500$ V, for measuring particles carrying a net positive charge. Two stable external power sources (DC Power Supply HP6516A and DC Power Supply RHR) supplied the analyzer's classification voltage. For each V_{CLASS} value, particle concentrations C_{INLET} and C_{OUTLET} were simultaneously measured with OPC1 and OPC2, respectively, for 30 s. Since both optical particle counters measure particles in sixteen size channels, a polydisperse aerosol can be analyzed by assuming a monodisperse aerosol fraction in each size channel. Thus, each aerosol fraction measured in an OPC2 size channel had a specific average electrical mobility corresponding to the applied classification voltage V_{CLASS} and the average particle diameter of that size channel. This electrical mobility can then be converted to the number of elementary charges, n, carried by these particles (Mainelis et al., 2001). The resolution in determining the number of electric charges, n, carried by extracted particles depends on the design characteristics of the electrical mobility analyzer. The analyzer used in this features parallel plate geometry, in which the width of the inlet channel, W_{INLET}, is equal to that of the outlet channel, W_{OUTLET} (Mainelis et al., 2001). Because of such geometry, the transfer function of

the device has a shape of an isosceles triangle and particles with critical mobility, $Z_{p,crit}$, are extracted with efficiency of 100% (Brown, 1997). For electrical mobilities that differ only incrementally from this critical value, less of the aerosol will be extracted. Since the transfer function has a shape of isosceles triangle, critical mobility, which corresponds to the peak of the triangle, is the same as the average electrical mobility of extracted particles. Thus, the maximum number of particles extracted will have an electrical charge equal to n.

By comparing C_{INLET} and C_{OUTLET} for the same size channel we determined the fraction, F_n, of particles with an average diameter equal to d_p, carrying n number of elementary charges at a given V_{CLASS}:

$$F_n(d_p, V_{CLASS}) = \frac{C_{OUTLET}(d_p, V_{CLASS})}{C_{INLET}(d_p)}. \quad (1)$$

By performing the same calculation for all V_{CLASS} values we determined the overall charge distribution for particles of diameter d_p at a specific aerosolization condition.

Our previous research (Mainelis et al., 2001) has shown that about 95% of *P. fluorescens* bacteria entering and leaving the classifier is concentrated in three size channels between 0.5 and 1.0 μm. A median of those three channels was selected to represent the bacterial diameter. The selected channel measures particles between 0.65 and 0.8 μm and its average diameter is 0.73 μm. *P. fluorescens* bacteria are about 0.7 μm in average optical diameter and 0.78 μm in average aerodynamic diameter as measured by Qian, Willeke, Ulevicius, Grinshpun, and Donnelly (1995), using an LAS-X optical particle size spectrometer (PMS Inc., Boulder, Colorado) and Aerosizer aerodynamic size spectrometer (Amherst Process Instruments Inc, Hadley, Massachusetts), respectively. Similarly, vast majority of *Bacillus subtilis* var *niger* spores measured upstream and downstream of the classifier lay within the range of 0.5–1.0 μm. Thus, for both bacteria, Eq. (1) was applied at the average diameter of $d_p = 0.73$ μm.

For the described configuration of the experimental setup and for the chosen d_p, the highest number of average elementary charges measured, 16550, corresponds to $V_{CLASS} = 5$ V, and the lowest number of average elementary charges measured, 18, corresponds to $V_{CLASS} = 4500$ V (Mainelis et al., 2001). The fraction of particles carrying less than 18 elementary charges, $F_{n,low}$, was determined by measuring the concentration of particles exiting the analyzer, C_{EXIT}, when a classification voltage of -4500 V was applied:

$$F_{n,low}(d_p = 0.73\ \mu m, V_{CLASS} = -4500) = \frac{C_{EXIT}(d_p = 0.73\ \mu m, V_{CLASS} = -4500)}{C_{INLET}(d_p = 0.73\ \mu m)}. \quad (2)$$

The overall charge distribution for each experimental condition was determined three times from which the average values and standard deviations were calculated. The electrical conductivity and pH level of each particle suspension were determined before, during and after the experiments using a conductivity meter and a pH meter.

The goal of this study is not only to determine the electrical charge distributions on aerosolized microorganisms but also to investigate how these charge distributions are affected by various factors. Since systematic error arising from the design of experimental apparatus is the same for all experiments, we believe that conclusions regarding those factors could be drawn without an exhaustive analysis of systematic errors.

3.4. Test particles

In this study we tested vegetative cells of *Pseudomonas fluorescens* and bacterial spores of *Bacillus subtilis* var. *niger* (BG) as representatives of biological particles and as well as sodium chloride (NaCl) particles as representatives of non-biological particles. The rod-shaped Gram-negative *P. fluorescens* bacteria are commonly found in ambient air (Nevalainen, 1989; Górny & Dutkiewicz, 1998) and represent sensitive bacteria (Neidhardt, Ingraham, & Schaechter, 1990). The rod-shaped Gram-positive BG spores are known to be very resistant to many adverse conditions (Sneath, 1986).

Dry BG spores were obtained from the US Army Edgewood Laboratories (Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland). Stock cultures of *P. fluorescens* (ATCC 13525) were obtained from the American Type Culture Collection (Rockville, Maryland). BG spores are rod-shaped, approximately 0.7–0.8 μm in width and 1.5–1.8 μm in length (Johnson, Martin, & Resnick, 1994). *P. fluorescens* vegetative cells range from 0.7 to 0.8 μm in diameter and 1.5–3 μm in length (Palleroni, 1984).

The BG spores were received in dry form and did not need additional cultivation. Prior to their aerosolization, a small amount of these spores was dissolved in deionized and sterilized 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, Maryland) at 30°C while kept for 18 h in a Gyrotory Water Bath Shaker (Model G76, New Brunswick Scientific Inc., Edison, New Jersey). The *P. fluorescens* cells were harvested from their suspensions by centrifugation at 5050 g for 7 min (Sorval RC-5B, Sorval Co., Newton, Connecticut). The resulting pellets were washed three times with deionized and sterilized water (5 Stage Milli-Q Plus System, Millipore Corp., Bedford, Massachusetts). To obtain suspensions of desired bacterial density, the initial suspensions of both microorganisms were diluted with deionized and sterilized water. The resistivity of used water is 18 MΩ cm (measured conductivity level is virtually 0 μS). The concentrations of *P. fluorescens* cells and BG spores in the air ranged from 400 to 800 particles/cm^3.

In the non-biological particle category, NaCl was chosen because it is frequently used in testing filters (Shu-Lai, Li-Wen, & Shian-Jang, 1996; Qian, Willeke, Ulevicius, & Grinshpun, 1997; Heikkinen, 2000). Sodium chloride particles were produced by aerosolizing 0.1% w/w NaCl solution, prepared by dissolving 1 g of reagent quality NaCl into 1 l of deionized and sterilized water. The measured conductivity of NaCl solution was 1650 μS.

4. Results and discussion

The data from our first set of experiments, Fig. 3, show that the airborne bacterial particles have electrical charge distributions which are very different from those of non-biological particles. In these experiments, *Pseudomonas fluorescens* bacterial cells, *Bacillus subtilis* var. *niger* bacterial spores and NaCl particles were aerosolized with the modified Collison nebulizer and their electric charge distributions were determined using Eq. (1). The x-axis in Fig. 3 represents the number of elementary electric charges, n, carried by the particles, while the y-axis shows the fraction of particles carrying n electric charges, F_n. Fig. 3A presents the entire measured charge range, while

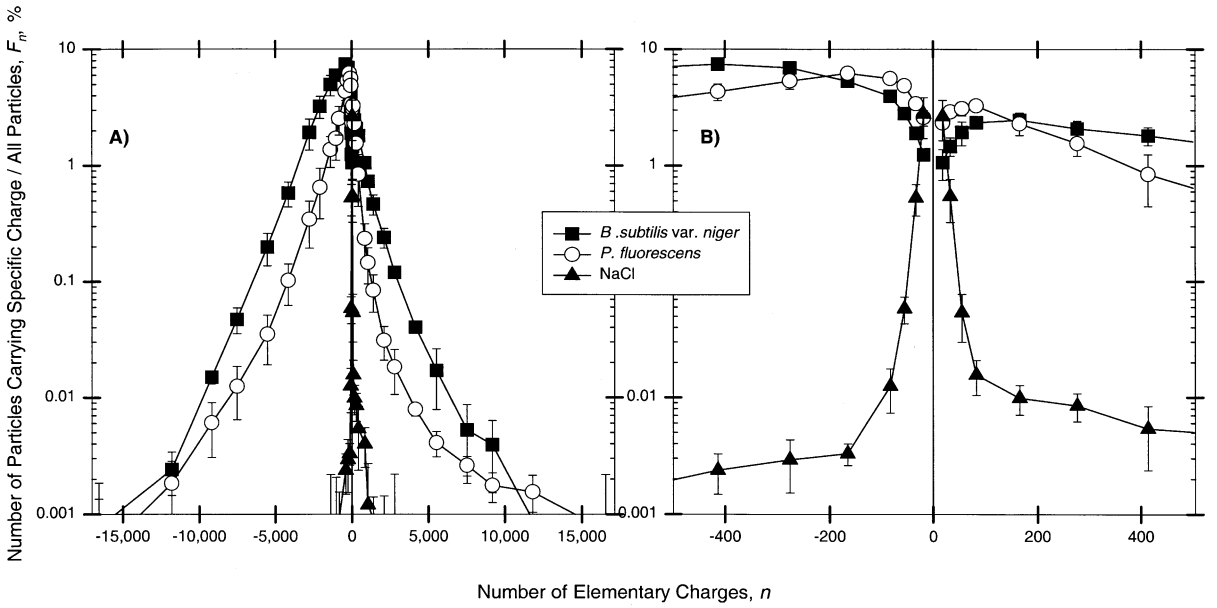

Fig. 3. Charge distributions of airborne *Bacillus subtilis* var. *niger*, *Pseudomonas fluorescens*, and NaCl, when aerosolized with a modified Collison nebulizer: (A) shown over wide elementary charge range, (B) shown near zero charge level. All particles sizes are 0.65–0.8 μm. Error bars indicate the standard deviation from the means of three repeats.

Fig. 3B shows details of the data near the zero charge level. The data presented in Fig. 3 show that both biological particles have a very wide electric charge distribution, with their net charge being negative. The fraction of BG spores carrying a specific number of elementary charges is higher than that of *P. fluorescens* over almost the entire measured charge range. However, the fractions of *P. fluorescens* bacteria carrying less than 200 elementary charges are higher than that of BG spores, Fig. 3B. We observed that a few bacteria of both bacterial species carried 13,000 or more elementary charges per bacterium. In contrast, the electric charge distribution of the NaCl particles of the same size is very narrow, ranging from a few hundred positive to a few hundred negative charges. From the shape of the charge distribution curve we can deduce that a major portion of the NaCl particles carries less than 20 elementary charges. The observed number of electric charges on airborne bacteria is also higher than that on 0.8 μm airborne latex particles as determined by Biermann and Bergman (1984). The workers aerosolized latex particles using a Wright-type nebulizer and reported that the highest particle count occurs at 14 elementary charge units, with some latex particles having as many as 1000 charge units.

In a second set of experiments, we examined how the different aerosolization methods affect the number of elementary charges carried by *P. fluorescens* vegetative cells. For this purpose, the *P. fluorescens* bacteria were aerosolized with the modified Collison nebulizer, the standard Collison nebulizer and the bubbling aerosol generator (Fig. 4). As seen, the net charge carried by the bacteria is negative for all three dispersion methods; however, the width of the electric charge distribution depends significantly on the dispersion method. The *P. fluorescens* bacteria carried the highest

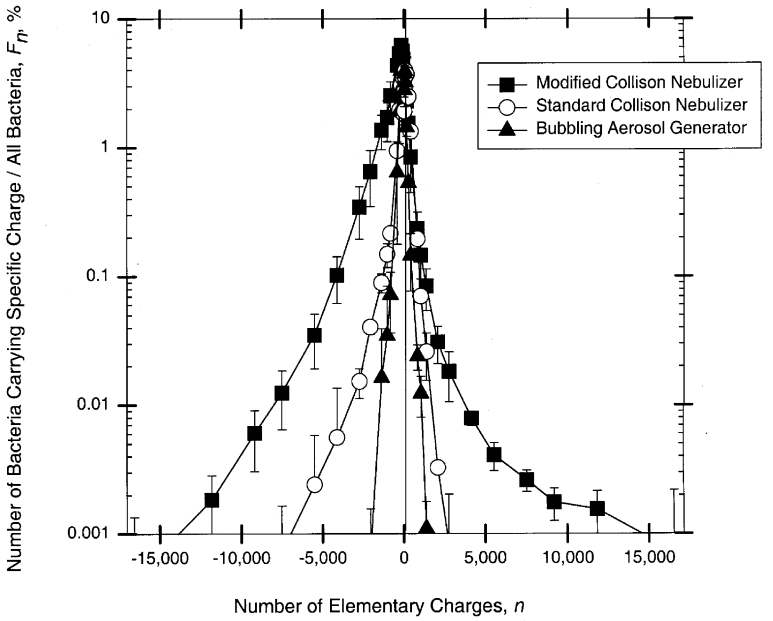

Fig. 4. Electric charge distribution on airborne *Pseudomonas fluorescens* bacteria (0.65–0.8 μm) when dispersed with a modified Collison nebulizer, a standard Collison nebulizer and a bubbling aerosol generator. Error bars indicate the standard deviation from the means of three repeats.

number of electric charges when dispersed by the modified Collison nebulizer. Some bacteria had electric charges as high as 13,000 elementary charge units. The bacteria aerosolized with the bubbling aerosol generator carried the fewest number of electric charges. Their overall charge distribution ranged from approximately 2000 negative to about 1000 positive charges. In this case, the bacteria still carried more elementary charges than the NaCl particles dispersed with the modified Collison nebulizer. For comparison purposes, we also examined electrical charges on NaCl particles aerosolized using the standard Collison nebulizer and the bubbling aerosol generator. This experiment (the results are not presented here) has shown that the NaCl particles carried from a few hundred positive to a few hundred negative electric charges when aerosolized with a standard Collison nebulizer and their charge distribution was somewhat narrower when aerosolized by a bubbling aerosol generator. Since the number of charges carried by the bacteria appears to depend on the dispersion method (Fig. 4) and some studies suggest that a spraying process itself imposes charges on droplets (Hendricks, 1973), we conclude that the measured charges on the bacteria are the sum of two charge components: their own natural charge and the charge imposed on them by the dispersion method. NaCl particles, in contrast, carry only the charges imposed on them by the dispersion process.

In a standard Collison nebulizer with 20 ml of water in the flask, most of the liquid solution is recirculated about every 6 s (May, 1973). In contrast, in the modified Collison nebulizer, large and heavy droplets settle at the bottom of the nebulizer and are drained. The drainage is not connected to the suspension being aerosolized and, thus, drained suspension is not rercirculated. However, once a generation cycle is complete, the drained suspension can be returned to the suspension feed

and dispersed again, thus performing a second generation cycle. In this manner, the number of generation cycles can be precisely controlled. By using this procedure, we examined the changes that occur in the charge distribution of *Pseudomonas fluorescens* bacteria, when the modified Collison nebulizer disperses the bacteria several times, see Fig. 5.

The curves in Fig. 5A show the electric charge distributions on *P. fluorescens* bacteria during the first three generation cycles. The curves in Fig. 5B compare the electric charge distribution during the fourth generation cycle with the charge distribution of the same bacteria after they have been washed by centrifugation immediately after the fourth generation cycle. These data indicate that the bacterial charge distribution becomes narrower with each additional generation cycle, i.e., bacteria carry fewer and fewer charges. However, after bacteria are washed by centrifugation, they have an electric charge distribution that is very close to that of the first generation cycle. This observation suggests that certain changes occur during repeated aerosolization that affect either the natural charge on the bacteria or the electric charge imposed on them by the dispersion process. In a standard Collison nebulizer the suspension undergoes many more generation cycles than in the modified nebulizer where the impaction wall is removed. Thus, we expect that the electric charge on bacteria may also undergo more significant changes.

This finding was examined further through the following experiment. The standard Collison nebulizer aerosolized *Pseudomonas fluorescens* bacteria for up to 180 min. During that time, we monitored bacteria carrying a net negative average charge of 2758 elementary units of charge (Fig. 6). The experiment was then repeated with a freshly prepared suspension of the same concentration. This time we examined bacteria carrying 827 net negative electric charges. In a similar manner, we monitored bacteria carrying 83 net negative elementary charges and less than 18 net negative elementary charges (-18 to 0). When monitoring the latter fraction of the bacterial charge distribution, we determined the ratio between C_{EXIT} and C_{INLET}, as shown in Eq. (2). The data presented in Fig. 6 show that over an aerosolization period of 180 min the fractions of bacteria carrying 2758 and 827 net negative elementary charges decreased about 8–10 times. In the latter fraction (carrying 827 net negative charges), the most profound changes occurred during the first 20 min of the nebulizer's operation. The fraction of bacteria carrying 83 net negative elementary charges did not change much over the time. The fraction carrying the least amount of charge (less than 18) increased by about 30–40% during the entire aerosolization period. These observations show that aerosolization of bacteria with a standard Collison nebulizer over a long period of time decreases the fractions of bacteria carrying high electric charges, while changes in the fractions of bacteria carrying low electric charges increase or stay the same.

In an effort to explain these observations we simultaneously monitored the conductivity, the pH levels of the suspension and the size distributions of the dispersed bacteria, Fig. 7. The results presented in Fig. 7A show that the aerosol concentration of droplet residues gradually increases, while the aerosol concentration of bacteria in the 0.65–0.8 μm size range slightly decreases, i.e., the size distribution of the aerosolized bacteria shifts toward smaller sizes. Terzieva et al. (1996) also noted that the airborne aerosol concentration of small particles increases with time when *P. fluorescens* bacteria are dispersed with a standard Collison nebulizer. During the dispersion time of 3 h, the electrical conductivity of the suspension increased from 0 to 40 μs, while the pH level increased from 6.4 to 7.1 (Fig. 7B). The pH level changed the most during the first 20 min of aerosolization and then its change was less profound. The conductivity of the suspension increased almost linearly with time.

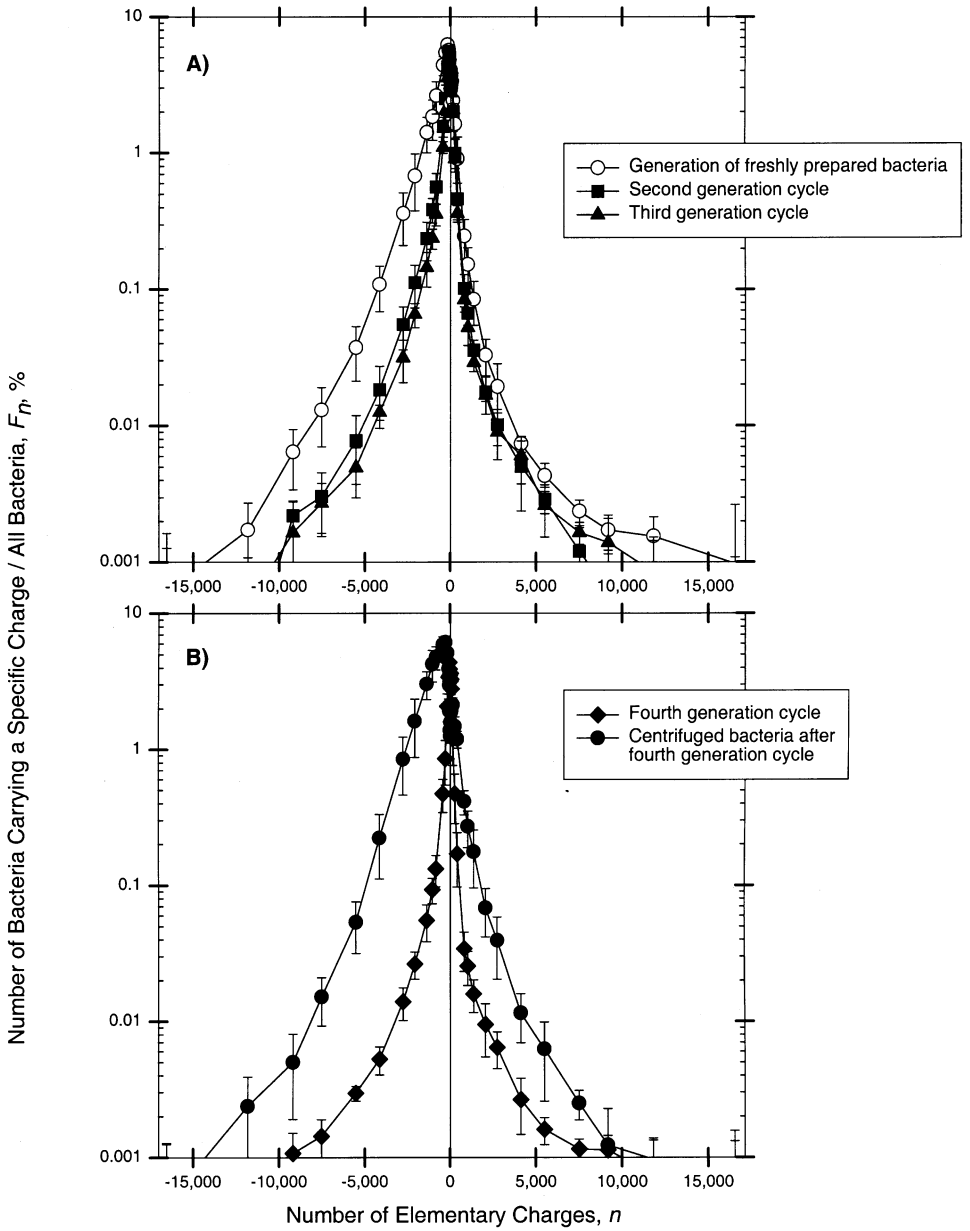

Fig. 5. Changes in the electric charge distribution on *Pseudomonas fluorescens* bacteria (0.65–0.8 μm) when the bacterial suspension is repeatedly aerosolized with the modified Collison nebulizer: (A) during first three generation cycles, (B) during the fourth generation cycle and when bacteria are washed by centrifugation. Error bars indicate the standard deviation from the means of three repeats.

This confirms our conclusion that the electric charge on airborne bacteria is likely to be the sum of two components: their natural charge and the charge induced by the spraying process. When bacteria are suspended in a growth medium (broth) or other electrolyte, their natural charge is

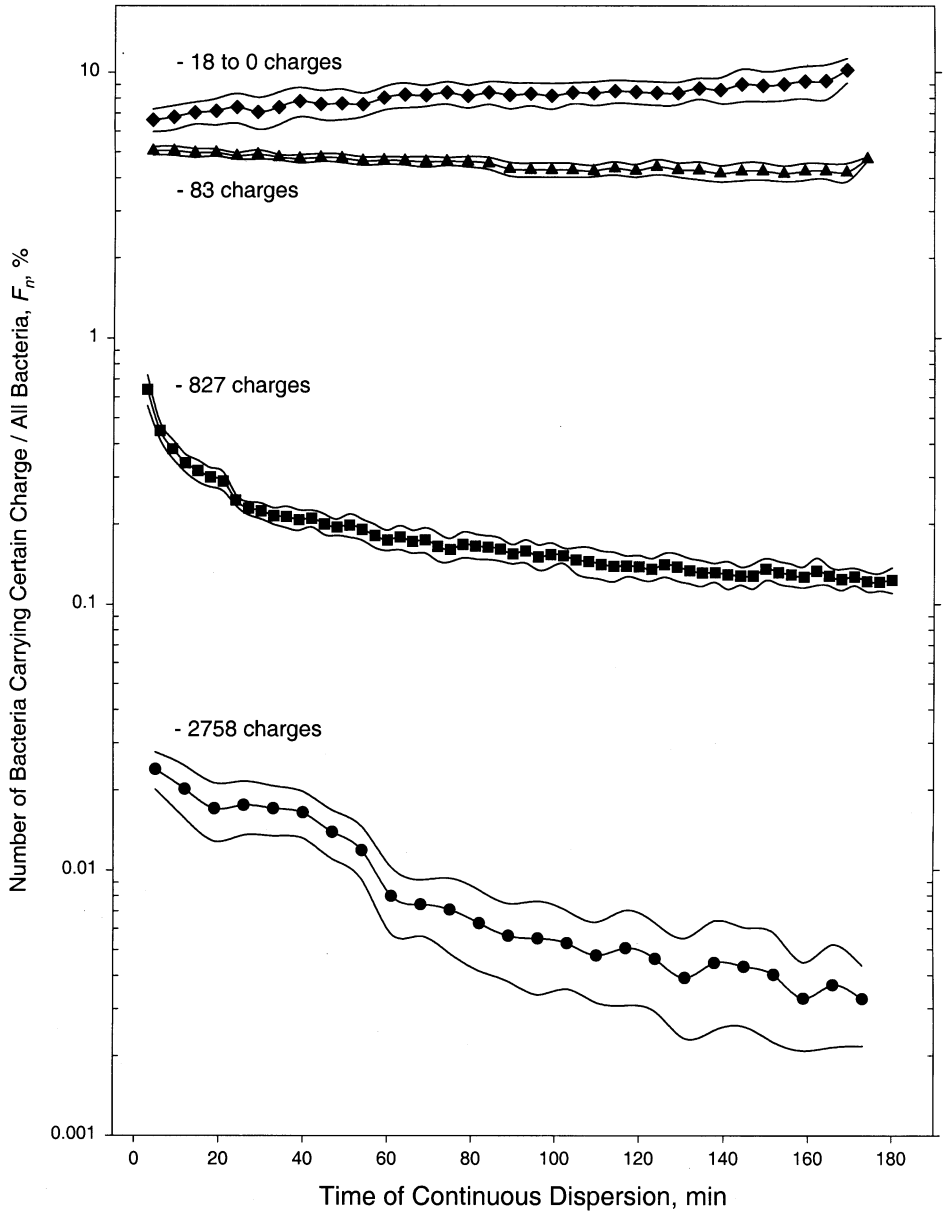

Fig. 6. Changes in the charge distribution of *Pseudomonas fluorescens* bacteria (0.65–0.8 μm) when dispersed with a standard Collison nebulizer. Symbols present the means of three repeats and the lines present 95% confidence intervals for the means.

counterbalanced by the formation of an electric double layer. As noted earlier, the ions of the first layer are strongly attached, while the ions in the second layer are diffuse and free movement is possible (James, 1979). When an external electric field is applied during electrophoresis, the bacteria and the firmly attached ions migrate to the appropriate electrode. The remaining counter-ions in the diffuse layer move in the opposite direction. When a bacterial suspension is prepared for

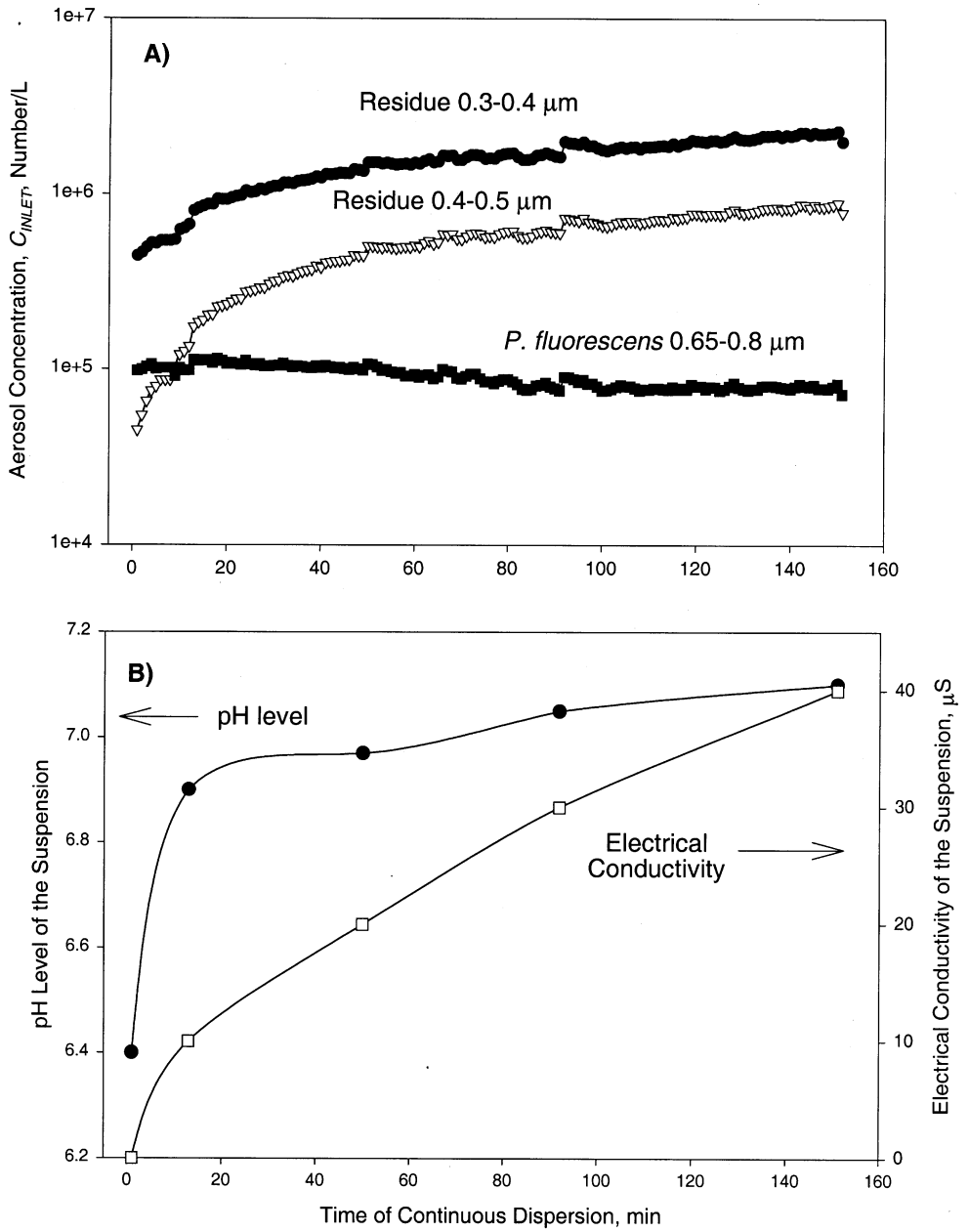

Fig. 7. Changes in suspension of *Pseudomonas fluorescens* bacteria when dispersed with a standard Collison nebulizer: (A) aerosol concentration, (B) pH level and conductivity.

aerosolization, the bacterial particles are removed from a liquid nutrient medium by means of centrifugation and then are suspended in deionized and filtered water. Since one part of the double layer is relatively loose, all of this layer or at least parts of it will be removed by centrifugation, thus

exposing charged bacterial components. This agrees with observation by Qian et al. (1995) also noted that washing, or centrifugation, may remove capsular material from the bacteria. In locations where the outer layer is removed, the bacterial surface charge is no longer counterbalanced. Since the electrical charge at the surface of bacteria is not consistently positive or negative over the entire surface or throughout the population (Aaronson, 1981), we expect that centrifugation will produce bacteria with both net positive and net negative charges. When these bacteria are then resuspended in deionized water, a new double layer starts forming. However, deionized water has very low conductivity (we measured 0 μS), which suggests that there are very few free ions that could participate in building such a layer and that the charge on the suspended bacteria will not be fully counterbalanced. Thus, when bacteria are washed, suspended in deionized water and then aerosolized, they will exhibit both negative and positive charges, in the airborne state.

The second part of bacterial charge comes from a dispersion process, which, as observed by Hendricks (1973), promotes spray electrification. However, the physical processes governing spray charging are still not well understood. Gu and Li (1998) have suggested that the charging mechanisms during droplet formation may be divided into three groups according to the charge origins. First, electric double layer charging or ionic charging occurs at the liquid–air interfaces. This charging mechanism is strong when the solution has a large number of dissociated ions. The second charging group is triboelectric charging, which arises from contact potentials since the liquid phase is always in contact with either solid surfaces or the ambient air during aerosolization. The triboelectrically charged liquid droplet dispersions may possess both positive and negative charges. The third charging group is due to the mechanical disruption of liquid surfaces in air when the droplets are formed. During this formation, the droplets obtain electric charges of certain magnitude and polarity. If there is a bacterium residing inside the droplet, the electric charge is transferred to the bacterium once the droplet has desiccated. Thus, the electric charge carried by a bacterium consists of two components: its own natural charge and the charge imposed onto it by the desiccated droplet. If these components are of the same polarity, the resulting bacterial charge will be higher; otherwise, the resulting charge will be lower.

The influences of different charging mechanisms on the net charge of aerosolized droplets depend on the process during which the droplet is formed (Gu & Li, 1998). As seen from Fig. 4, *Pseudomonas fluorescens* bacteria aerosolized with the bubbling aerosol generator carry fewer charges than bacteria dispersed with the modified or standard Collison nebulizer. The liquid disrupting forces, or triboelectric forces, inside the bubbling aerosol generator are weaker than those in the compressed air dispersers (Collison nebulizers). Thus, the droplets and the bacteria that remain airborne after drying carry fewer charges when aerosolized by the bubbling aerosol generator than when aerosolized by the compressed air dispersers.

When *P. fluorescens* bacteria are repeatedly dispersed with a modified Collison nebulizer, they carry fewer and fewer charges, as seen in Fig. 5. The compressed air inside the stem of a Collison nebulizer develops a strong force for disrupting the liquid suspension. We postulate that the fragments of charged bacterial surfaces are shaved-off when these violent forces disperse bacteria. Terzieva et al. (1996) speculated that shear forces in a standard Collison nebulizer may break off bacterial slime or cell walls. Once these fragments are removed from the surface of an airborne bacterium, the resulting net charge becomes higher or lower, depending on the electric charges carried by the now-missing fragments. The fragments produced during aerosolization either become airborne or are drained. Once in the drained suspension, these fragments contribute to the

conductivity of the suspension. Some of the fragments may recombine with bacteria altering their charge again. This process is repeated with each generation cycle. Thus, the amount of physical stress endured by bacteria during their aerosolization may be determined from the changes in magnitude of electric charge carried by these bacteria. After four generation cycles, the conductivity of the suspension increased from 0 μS (initial suspension) to 10 μS. However, even after four generation cycles with the modified Collison nebulizer (Fig. 5B, solid diamonds) the *P. fluorescens* bacteria carried more charges than when they were aerosolized with a standard Collison nebulizer (Fig. 4, open circles). When, after four generation cycles, the suspension was washed and the bacteria were resuspended in fresh deionized water, the conductivity of the suspension was again 0 μS, i.e., the conductive fragments were removed by the wash cycle. The bacteria dispersed from the reconstituted suspension had a charge distribution close to that of the freshly prepared bacteria (Fig. 5B, solid circles).

These effects are very pronounced when the standard Collison nebulizer disperses bacteria over a long period of time, Fig. 6. The electric charge imposed on the bacteria by the dispersion process depends on the size of the droplets containing these bacteria. Since the droplets emitted by the standard Collison nebulizer are smaller than those produced by the modified Collison nebulizer, the electric charge distribution on bacteria dispersed by the latter device is wider than that on bacteria dispersed by standard Collison nebulizer, as seen in Fig. 4. Also, as seen from Figs. 6 and 7, the bacterial suspension inside the standard Collison nebulizer undergoes significant changes. Since the bacteria pass through the same type of nozzle as in the modified Collison nebulizer, fragments of bacterial surfaces are also shaved off and contribute to the electrical conductivity of the suspension. Significant increases in the electrical conductivity of the suspension are seen in Fig. 7B. Strong shear forces significantly increase the number of fragments in the suspension. This increase can also be observed from the increased aerosol concentration of residue particles (0.3–0.5 μm particles) as seen in Fig. 7A. During the same time, the pH level of the suspension increases (Fig. 7B), while the fractions of bacteria carrying high electric charges decrease (Fig. 6). As a consequence, the fraction of bacteria carrying low electric charges increases. According to Gittens and James (1963), the average electric potential of a liquid-borne bacterium at its separation layer is a direct function of the ionogenic surface charge, modified by the presence of ions in the suspension. Thus, changes in the electric charge distribution of airborne bacteria may be caused by three factors: removal of conductive fragments from the bacterial surface by shear forces of dispersion, increase in the conductivity of the suspension, and increase in the pH level of the suspension. Since the increase in the number of fragments in bacterial suspension coincides with the increase in the conductivity and pH level of the suspension, we believe that the latter two parameters may be used as indicators of the mechanical stress, which results in the injury and loss in viability, endured by bacteria during aerosolization.

When particles are dispersed with a standard Collison nebulizer, they may frequently collide with each other. According to Forsyth, Liu, and Romay (1998), particle collisions during dispersion may alter their electric charge.

The described processes show that the electric charges carried by airborne bacteria depend on a variety of physical factors and mechanisms. The precise role of these factors or mechanisms is yet to be studied. However, even when *Pseudomonas fluorescens* bacteria were dispersed by a bubbling liquid (a gentle aerosolization method) they carried up to 2000 charges per bacterium. Since airborne microorganisms can carry high electric charges, their collection by electrical field forces

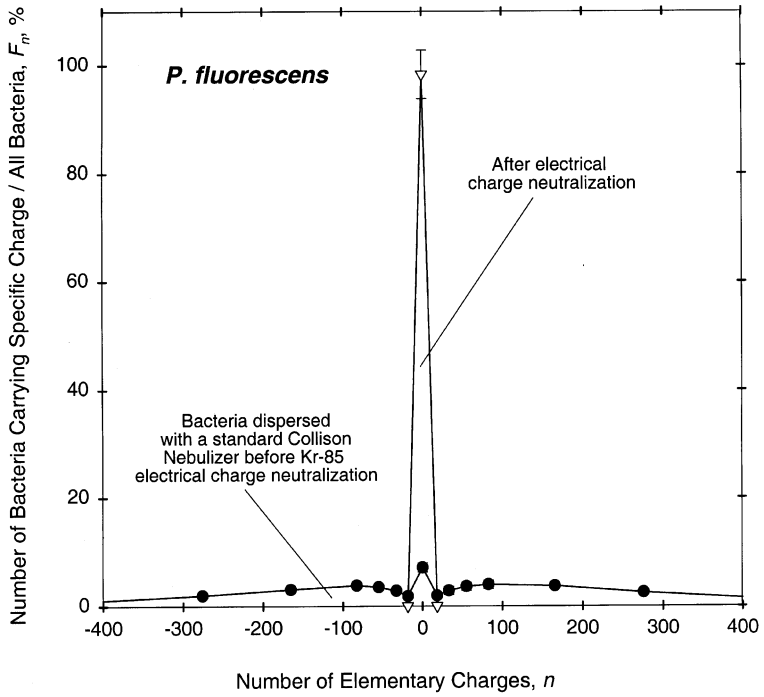

Fig. 8. Effect of radioactive Kr-85 neutralization on the electric charge distribution of *Pseudomonas fluorescens* bacteria (0.65–0.8 μm). Error bars indicate the standard deviation from the means of three repeats.

may be possible without first electrically charging them. Since the amount of electric charge on aerosolized bacteria depends on the method of aerosolization, the sampling may be affected by the way in which the bacteria are released into the air environment.

In a natural environment, it is likely that the electrical charges on airborne microorganisms are affected by free atmospheric ions or by natural radiation. We simulated the effect of radiation on the bacterial charge by passing *P. fluorescens* bacteria through a 10mCi Kr-85 electrical charge neutralizer following their aerosolization by a standard Collison nebulizer. From the data shown in Fig. 8 we can see that the *P. fluorescens* bacteria carry less than 18 elementary charges after passage through the charge neutralizer, i.e., the bacteria are almost completely neutralized by the Kr-85 source. In the normal atmosphere, cosmic rays and other radioactive elements produce bipolar ions in concentrations of 500–1000 ion pairs/cm^3 of air (Yeh, 1993). At such bipolar ion concentrations particles loose approximately half of their electric charge in 5–10 min (Hinds, 1999). Thus, in a natural environment, airborne bacteria may carry fewer electric charges than their laboratory-aerosolized counterparts.

5. Conclusions

Airborne particles are usually characterized by their concentration and physical size. Our research has shown that particles in the airborne state may also be characterized by the magnitude

and polarity of their electric charges. Since biological and non-biological particles were found to differ in the amount of electric charge carried, this parameter may be used to separate airborne biological particles from their non-biological counterparts. When vegetative cells of *Pseudomonas fluorescens* are repeatedly aerosolized through a Collison nebulizer's orifice, the electric charge distribution of these sensitive bacteria undergoes significant changes which we postulate to be caused by the shear forces of dispersion. Thus, the amount of stress endured by bacteria during their aerosolization may be determined from the magnitude of electric charge carried by these bacteria. Differences in electric charge level, depending on the aerosolization method, may affect the data, e.g., when sensitive bacteria, such as *P. fluorescens*, are used as challenge particles in filter testing. The magnitude of electric charge carried by these particles changes every time when they pass through a Collison nebulizer's nozzle and that, in turn, may affect their efficiency of collection. Therefore, when testing particle collection on filters, aerosolization methods that induce the least amount of changes on the challenge particles should be used. We also found that the electrical conductivity and the pH level of the bacterial suspension increase during aerosolization by a Collison nebulizer. Thus, these two parameters may be used as indicators of the mechanical stress, injury and loss in viability, endured by bacteria during aerosolization, i.e., measuring the electrical conductivity and pH level of bacterial suspensions may be a simple and convenient method for monitoring the "wear and tear" of the bacteria suspended in deionized water. This study has shown that the release method and the presence of bipolar ions can affect the magnitude of electrical charge on aerosolized bacteria. Thus, in natural environments, where the microorganisms aerosolized by various methods are affected by background radiation, bioaerosol particles may carry electric charges different from that of their laboratory-aerosolized counterparts.

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