AIRBORNE MICROORGANISM COLLECTION BY A NEW ELECTROSTATIC PRECIPITATOR

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ABSTRACT
Many aerobiological measurements and the protection of governmental/military establishments from bioterrorism require the development of new bioaerosol collectors that can be operated efficiently at low power. We have developed and evaluated a new bioaerosol sampler in which the microorganisms are collected by electrostatic means. An ionizer charges the incoming microorganisms, if they carry insufficient charge for efficient collection in the device. The microorganisms are collected on two square agar plates placed along the flow axis. Laboratory experiments have shown that bacterial cells and spores are generally collected more efficiently than inert particles. We attribute this difference to the natural negative charge contained in the cell membranes of microorganisms. The results of field measurements parallel those obtained through laboratory experiments.

INDEX TERMS
Collection efficiency, airborne microorganism, electrostatic precipitation, electrical charge, bioaerosol sampler.

INTRODUCTION
Airborne microorganisms in residential and occupational indoor air environments may cause various respiratory and other health disorders in individuals exposed to them (Burge, 1990; Schachter, Maunder and Beck, 1984). The most common method for assessing the exposure concentration of bioaerosols is by use of impaction or impingement devices. The particle velocity towards the collection medium in these devices is usually tens or hundreds of meters per second, which results in high collection efficiency, but potentially also in loss of viability in the more sensitive microorganisms (Stewart, Grinshpun, Willeke, *et al*., 1995; Lin, Reponen, Willeke, *et al*., 2000). We have studied the electrostatic properties of airborne microorganisms and the use of electrostatic forces for the collection of microorganisms in order to develop a more gentle technique for bioaerosol collection. Since the particle velocity component perpendicular to the collection medium in an electrostatic precipitator is two to four orders of magnitude lower than that in bioaerosol impactors and impingers at comparable sampling flow rates (Mainelis, Grinshpun, Willeke, *et al*., 1999), the electrostatic precipitation technique is potentially less damaging to the microorganisms and can be used at low power input. This is of interest not only to field practitioners, but also to individuals planning the placement of low power consuming monitors in and around buildings and installations as warning devices against bioterrorism.

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In a conventional electrostatic precipitator, used for the collection of non-biological particles, the aerosol is drawn past a corona discharge, which generates air ions. Many of the air ions attach themselves to particles while crossing the aerosol flow through the inlet. The charged particles are then removed from the airflow by passage through an electrostatic field. In our first attempt to use the electrostatic precipitation technique, we modified a conventional electrostatic precipitator for bioaerosol collection on agar plates. We found that hardy microorganisms, such as *Bacillus subtilis* var. *niger* spores, can be efficiently collected by this technique; however, sensitive microorganisms, such as vegetative cells of *Pseudomonas fluorescens*, suffered significant losses in viability during the collection process (Mainelis, Grinshpun, Willeke, *et al*., 1999). We attributed this loss in viability to the corona discharge.

In our subsequent research we found that the electrical charging of microorganisms is a rather complex process and that several factors need to be taken into account when designing a new bioaerosol collector based on electrostatic techniques (Mainelis, Willeke, Baron, *et al*., 2001). Taking into account our findings on the electro-biological properties of airborne microorganisms, we have designed, built and tested a new electrostatic precipitator for bioaerosol collection.

DESIGN OF THE ELECTROSTATIC PRECIPITATOR

The device consists of three distinct components: the charging section, plates filled with the collection medium (e.g. agar), and the precipitation section. We found that airborne microorganisms may carry very high electrical charges upon their dispersal into air, particularly when dispersed through conventional laboratory generators. With time, atmospheric radiation may significantly decrease the amount of electrical charge on the airborne microorganisms (Mainelis, Willeke, Baron, *et al*., 2001). Many airborne microorganisms appear to naturally contain electrical charges in their outer shell. E.g., some of the airborne organic particles in Antarctica have been found to be positively charged, with their deposition to the ground influenced by their charge in the natural electric fields (Benninghof and Benninghof, 1982). Electrical charging of the microorganisms may, therefore, not be necessary, depending on when and where the measurements are made. For monitoring in environments where the electrical charges on the airborne microorganisms are low, we have included two commercially available air ionizers (AS150, Wein Products, Inc., Los Angeles, CA) in the inlet section of the electrostatic precipitator. If the ionizers are activated, they project air ions across the inlet flow and add electrical charges to the microorganisms. The charging intensity can be adjusted by varying the ionizers' voltage. These air ionizers produce vitually no ozone, which appears to be the principal cause for loss in viability when the airborne microorganisms are electrically charged by corona discharge, the conventional charging mechanism in electrostatic precipitators.

After passage through the inlet/charging section the microorganisms enter the precipitation section, where they are collected onto two square Petri dishes that are placed one after the other in the flow direction. The Petri dishes are usually filled with agar for subsequent incubation, so that the colony-forming units can be enumerated. A low-power, battery-operated fan establishes the airflow. Batteries can also operate the electrical components.

METHODS

The new device has been evaluated in the laboratory and in the field. In the laboratory experiments, the device was first evaluated as to its physical collection efficiency. The principal components of the experimental setup for these measurements were a three-hole Collison nebulizer (BGI Inc., Waltham, MA) for dispersing the microorganisms into air, a Kr-

85 charge neutralizer (model 3054, TSI Inc., St. Paul, MN) for reducing the electrical charge on the airborne microorganisms to Boltzmann equilibrium prior to entering the electrostatic precipitator, and two identical optical particle counters (model 1.108, Grimm Technologies Inc., Douglasville, GA). The physical collection efficiency was determined from the ratio of the particle count downstream of the electrostatic precipitator to the concentration measured upstream of the device's inlet. The entire test system was placed in a Class II, Type B2 biological safety cabinet (SterilchemGARD, Baker Company, Sanford, ME).

The biological collection efficiency was determined in laboratory experiments by comparing the colony-forming units enumerated on the Petri dishes after incubation relative to the number of colony-forming units enumerated from a BioSampler (SKC Inc., Eighty Four, PA) placed near the inlet of the new electrostatic precipitator. The test particles for the physical and biological collection efficiency determinations were: sodium chloride (NaCl), *Bacillus subtilis* var. *nige*r (BG spores and cells), *Pseudomonas fluorescens*, and *Penicillium brevicompactum*. The rod-shaped Gram-positive BG spores are known to be very resistant to many adverse conditions (Sneath, 1986), while the vegetative cells of the same species have been shown to be more sensitive to the same stress (Yonemoto, Yamaguchi, Okayama, *et al.*, 1992). During the laboratory experiments the air temperature was maintained at 22-26°C and the relative humidity at 30-50%.

The field experiments were first performed in indoor air. By dispersing bacteria and fungal spores from carpets under controlled conditions, the new electrostatic precipitator was evaluated as to its optimal charging condition (including charging polarity) and precipitation voltage. Subsequent measurements were performed in an agricultural environment and in an industrial facility with bacteria-containing cutting fluids dispersed into the air.

All data presented below represent the average of three repeats. The data analysis was performed using analysis of variance (ANOVA) available as an add-in to Microsoft Excel 2000. P values of <0.05 were considered significant.

RESULTS

In our first set of laboratory experiments, we determined the overall collection efficiency of the new device, when sampling "charge-neutralized" NaCl particles at different flow rates with no additional charging applied. The collection efficiency increased with increasing

Table 1. Overall collection efficiency of *Bacillus subtilis* var. *niger* (BG) spores at different flowrates and precipitation voltages.

Flowrate	*Overall Collection Efficiency, %*						
L/min	*-5,000V*	*-3,000V*	*-1,000V*	*0V*	*+1,000V*	*+3,000V*	*+5,000V*
2	87	79	40	0	45	78	87
4	77	56	20	0	28	67	79
8	54	35	14	0	9	30	48

positive or negative precipitation voltage. It also increased with decreasing flow rate through the device. Similar experiments were performed with *Bacillus subtilis* var. *niger* vegetative cells and spores. The collection efficiencies for comparable precipitation voltages and flow rates were considerably higher. At a flow rate of 2 L/min, about 50% of the biological particles were removed from the air at a positive or negative precipitation voltage of 1,000 V.

The collection efficiency increased to about 90 %, when the positive or negative precipitating voltage was increased to 5,000 V. Table 1 shows an example for BG spores. When the sampling flow rate was doubled to 4 L/min, more than 75 % of the biological particles were still collected with positive or negative precipitation voltages of 5,000 V.

When electrical charging was added, the physical collection efficiencies increased significantly for biologically inert and biologically active particles. Some of the data at a specific charge level are shown in Table 2. At a flow rate of 4 L/min, application of a low precipitation voltage of about 200 V resulted in the collection of 50% of the NaCl particles. 90 % or more of the NaCl particles were removed from the air at a positive or negative precipitation voltage of 5,000 V. Vegetative cells and spores of *Bacillus subtilis* var. *niger* were removed at 70 % efficiency with the application of a positive or negative precipitation voltage of 200 V. For the cells and spores, a collection efficiency of about 90 % was achieved with the application of a negative precipitation voltage of 2,000 V. To achieve the same physical collection efficiency with a positive precipitation voltage, the precipitation voltage had to be between 4,000 and 5,000 V.

Table 2. Overall collection efficiency of NaCl particles and *Bacillus subtilis* var. *niger* (BG) spores and vegetative cells, sampled at 4 L/min and exposed to two ionizers at 1.3V (115mA).

Test Particle	*Overall Collection Efficiency, %*						
	-5,000V	*-3,000V*	*-1,000V*	*0V*	*+1,000V*	*+3,000V*	*+5,000V*
NaCl, 0.73μm	98	95	81	0	66	80	90
BG veg. cells	99	95	83	0	78	88	93
BG spores	99	97	90	0	76	92	94

In the next set of experiments (at 4 L/min), the biological collection efficiencies, (No. of colony-forming units on agar plates)/[(% viability in liquid suspension)(No. of aerosolized bacteria entering sampler)], were determined for *Pseudomonas fluorescens*, *Bacillus subtilis* var. *niger*, and *Penicillium brevicompactum* by comparing the colony-forming units on the agar plates after incubation with the number of colony-forming units enumerated from collection in a BioSampler (SKC Inc., Eighty Four, PA). Table 3 shows some biological collection efficiency data for *Penicillium brevicompactum* fungi, which are particularly difficult to aerosolize, resulting in significant standard deviations. When no electrical charging was applied in the inlet, the biological efficiency relative to the BioSampler for *Penicillium brevicompactum* was about 100 % at a negative precipitation voltage of 4,000 V. At a positive precipitation voltage of 4,000 V, however, the relative biological collection efficiency was only about 10 %. When the incoming microorganisms were positively charged, the biological collection efficiency relative to the BioSampler was again about 100 % at a positive precipitation voltage of 4,000 V.

The results of the field experiments paralleled the results of the laboratory experiments. The first field experiments were performed in indoor air. When bacteria and fungal spores were dispersed into air by a vacuum cleaner, operated in reverse flow with or without a beater bar, the aerosolized microorganisms were initially highly charged, so that electrical charging was not necessary for the collection of the microorganisms in the electrostatic precipitator. With time, however, the electrical charge decreased due to atmospheric radiation, thus decreasing the collection efficiency, when no electrical charging was applied in the inlet. Additional field experiments were performed in an agricultural environment and in an industrial facility involving aerosolized bacteria contained in continuously recycled metal cutting fluid droplets.

Table 3. Biological collection efficiency of *Penicillium brevicompactum*, sampled at 4 L/min in the new electrostatic precipitator, and at 12 L/min in the BioSampler.

Sampler	*Precipitation Voltage*	*Biological Collection Efficiency, %*	
		No Ionization	*+1.25V Ionization*
Electrostatic Precipitator	*-4,000V*	94 ± 23	4 ± 4
	-1,300V	31 ± 19	4 ± 6
	+1,300V	4 ± 6	75 ± 40
	+4,000V	10 ± 5	75 ± 27
BioSampler		78 ± 35	78 ± 35

DISCUSSION

A cloud of inert particles suspended in air generally has a net neutral charge. However, individual particles may have positive or negative charges according to the Boltzmann charge distribution (Flagan, 2001). Thus, many or most of the particles in a net neutral cloud of particles can be collected in an electrostatic field, if the field is sufficiently strong. This is why our experiments had significant physical collection efficiencies even without electrical charging at the inlet.

Our earlier experiments with bioaerosols have shown that a cloud of airborne microorganisms tends to have a net negative charge. We attribute this to electrical charges that are naturally present in the outer shells of these microorganisms. Bioaerosol collection in a negative electric field, therefore, appears to be more efficient (the agar being at ground potential). If additional charging is applied, the charging should be negative, if the precipitation field is negative, or the charging should be positive, if the precipitation field is positive. Otherwise, the microorganisms will migrate away from the agar plates towards the top of the collector, i.e. they will be collected in the device, but not on the agar plates. When the flow rate is reduced, the particles have a longer residence time in the device and are, therefore, more likely to be removed from the airflow by the electrostatic field. Thus, collection efficiency is inversely related to flow rate.

CONCLUSION AND IMPLICATION

The data prove that the newly designed and built electrostatic precipitator is capable of gently collecting airborne microorganisms. A small amount of ionization in the device enhances the overall collection efficiency. The tested microorganisms carried a net negative charge. Thus, if no additional charging is applied, these microorganisms should be collected using a negative precipitation field so that they collect on the collection surface (usually agar) and not on the other electrode. Since very little power is needed for bioaerosol collection by this method, it is attractive for field sampling and low-power monitoring networks.

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