Final Performance Report

NIOSH Grant 5 R01 OH003463-03, Years 1 through 3
"Electrostatic Sampling of Airborne Microorganisms"

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Abstract

Each year millions of respiratory allergies and infections are caused by airborne microorganisms present in agricultural, industrial and indoor environments. The level of exposure indicated by bioaerosol samplers depends on the instrument used and the sensitivity of the microorganisms. In an effort to collect such microorganisms more gently, at low power and at minimal pressure drop, an electrostatic sampling technique has been developed and evaluated. As a major part of this development, an electrostatic particle-size classifier and a microorganism dispersion device with optional induction charging were developed to study the electric charges on airborne microorganisms. It has experimentally been proven that laboratory-dispersed indoor air bacteria, such as *Pseudomonas fluorescens*, have a net negative charge. Some of the bacteria were found to carry several thousand negative or positive charges. In contrast, particles of non-biological origin were found to carry very few positive or negative charges. This finding suggests that the electrostatic sampler may retain airborne microorganisms by its electrostatic collecting field without first charging the microorganisms in the inlet section, thus reducing the complexity and power consumption for sampling in occupational environments.

Significant Findings

The overall objective of this research was to use electrostatic mechanisms for the efficient collection of airborne microorganisms in a nutrient medium, and to develop a prototype electrostatic bioaerosol sampler. These objectives have been met: Several studies were undertaken on the behavior of airborne microorganisms in an electrostatic filed and a prototype electrostatic bioaerosol sampler has been developed.

Through this research, it was found that airborne microorganisms may naturally carry significantly higher levels of electrical charge than non-biological particles, particularly when dispersed from a liquid. This implies that one may be able to collect such microorganisms without first charging them. For instance, when working with metalworking fluids containing microorganisms, one would expect a high level of electric charge on the airborne microorganisms.

In order to perform laboratory tests on airborne microorganisms, a bioaerosol generator was developed that gently imposes electric charges on the microorganisms by electric charge induction. Conventional electrical charging by corona discharge is known to kill sensitive microorganisms.

In order to study the viability of airborne microorganisms carrying a specific positive or negative electrical charge, an electrical charge classifier was developed. It was found that even sensitive microorganisms can maintain a high negative charge without loosing their viability. However, high positive charges may kill them. We attribute this to naturally present negative charges in the cell walls of the microorganisms.

Usefulness of Findings

Each year millions of respiratory allergies and infections are caused by airborne microorganisms present in agricultural, industrial and indoor environments. The findings from this research grant are being used to develop more efficient and less power consuming sampling methods for the collection of airborne microorganisms, while maintaining their viability. The findings also give us a better understanding of the behavior of microorganisms when suspended in air.

Peer-reviewed Journal Articles that Have Resulted from this Grant (with Acknowledgement to the Grant. Copies of the Articles are Attached to this Report)

Mainelis G, Grinshpun SA, Willeke K, Reponen T, Ulevicius V, Hintz PJ: Collection of Airborne Microorganisms by Electrostatic Precipitation. Aerosol Science and Technology 30:127-144, 1999

Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Induction Charging and Electrostatic Classification of Micrometer-Size Particles for Investigating the Electrobiological Properties of Airborne Microorganisms. Aerosol Science and Technology, accepted for publication, 2001

Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Electrical Charges on Airborne Microorganisms. J. of Aerosol Science, accepted for publication, 2001

Mainelis G, Gorny R, Willeke K, Reponen T, Grinshpun SA, Yadav J, Baron PA: Effect of Electrical Charges and Fields on Viability and Injury of Airborne Bacteria. Biotechnology and Bioengineering, submitted, 2001

Proceeding Article (with Acknowledgement to the Grant)

Willeke K Mainelis G, Grinshpun SA, Reponen T, Baron P: Measurement of Electrical Charges on Airborne Microorganisms, Journal of Aerosol Science 31: S957-958, 2000

The research results have also been presented at the annual American Industrial Hygiene Conferences in 1998 (Atlanta), in 1999 (Toronto), in 2000 (Orlando) and in 2001 (New Orleans, at the annual American Association for Aerosol Research Conferences in 1998 (Cincinnati) and in 1999 (Tacoma), and in 2000 at the European Aerosol Conference (Dublin).

Research Results Contained in the Journal Articles

First Article: Mainelis G, Grinshpun SA, Willeke K, Reponen T, Ulevicius V, Hintz PJ: Collection of Airborne Microorganisms by Electrostatic Precipitation, Aerosol Science and Technology 30:127-144, 1999.

Before designing a new electrostatic sampler for bioaerosol collection, we decided to first gain experience with the electrostatic precipitation of bacteria by modifying and using a commercially available electrostatic aerosol sampler that was developed for the collection of non-biological particles. We acquired an Electrostatic Aerosol Sampler (EAS) (model 3100, TSI Inc., St. Paul, MN), consisting of an inlet, a flow straightener (honeycomb), a corona charging section, an electrostatic precipitation section and an outlet. In order to use this device for the collection of bacteria, a 0.8 cm deep, 4.8 cm wide and 18 cm long well was milled into the collection area so that a rectangular trough could be placed inside. The trough was filled with agar or water for collecting and subsequently incubating bacteria. We also used a filter as the collection medium; in this case, a conducting support was placed into the trough underneath the filter to ensure that the collection surface was at the same height as when sampling with agar or water. The electric field in the precipitation section was 4.2 kV/cm; the corona discharge in the charging section was produced by a 0.05 mm diameter tungsten wire maintained at a voltage of 3.5 kV.

The major findings of relevance to the development of an electrostatic bioaerosol sampler were as follows. Tests conducted with three bacterial species and with agar, water, and filter as collection media have shown that the electrostatic precipitation method is suitable for collecting viable airborne microorganisms. When collecting *Bacillus subtilis* var *niger* (BG) spores on agar, about 50 to 60 % of the collected culturable microorganisms formed colonies in the EAS. However, a much lower recovery rate was obtained for *Pseudomonas fluorescens* and *Mycobacterium bovis* BCG vegetative cells, which suggests that these bacteria may have sustained injuries in the charging section through the corona discharge (which produces ozone and nitric oxides). Additional embedding of the collected bacteria into agar did not result in an increase of the bacterial recovery rate. When the EAS pump was turned off and tests were performed with the three bacterial species inoculated on agar or in water in the collection section (i.e., no prior exposure to corona discharge) while exposing them to 4.2 kV/cm, the culturability was not reduced during test periods of up to two hours. We concluded from these data that even sensitive bacteria, such as *Pseudomonas fluorescens*, were not inactivated by the electric field after their collection on agar or in water. This finding encouraged us to find ways to avoid the use of corona discharge in the charging section and to find ways of measuring the electric charge distribution on airborne microorganisms.

Second Article: Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Induction Charging and Electrostatic Classification of Micrometer-Size Particles for Investigating the Electrobiological Properties of Airborne Microorganisms, Aerosol Science and Technology, accepted for publication, 2001.

This article deals with the development of an aerosol generator with electric charge induction and an aerosol electric charge classifier. The development of the aerosol generator with charge induction was necessary so that we would have a means for aerosolizing particles of biological and non-biological origin with or without specific charge levels imposed on them. We could have dispersed the microorganisms by more conventional means and then charged them by corona discharge. However, that would have rendered too many of the microorganisms non-viable, as documented in our first article. Therefore, we developed a more gentle method by charging the microorganisms through induction. While induction charging has been successfully performed in a modified vibrating orifice aerosol generator for the generation of particles from a liquid solution, we are not aware of any such device for the dispersion of microorganisms from a suspension and their simultaneous induction charging.

In the new aerosol generator, the microorganisms are dispersed through the center stem of a Collison nebulizer (BGI Inc., Waltham, MA), i.e., the housing of the Collison nebulizer acting as the impaction surface is removed. After dispersion by the Collison's sonic orifice air jet, the liquid-borne microorganisms pass through a second, larger orifice, surrounded by air, which separates the particles from each other due to the pressure drop across the second orifice. Addition of dry air evaporates the water so that only airborne microorganisms and much smaller droplet residues leave the aerosol generator. Large droplets settle to the bottom of the generator and are drained. When a positive or negative voltage of up to 3,000 volt is applied to the stainless steel Collison stem, while the second orifice is grounded, electric charges are induced onto the droplets as they are being formed from the liquid exiting the liquid feed tube in the Collison stem.

The charged bioaerosol particles then enter our newly developed aerosol electric charge classifier. The bioaerosol particles enter through a channel, which is parallel to clean sheath air. By applying a voltage potential across this parallel plate device, the bioaerosol particles carrying a desired electric charge range are extracted.

During the first few months of working with these new devices in our test facility we found that many of the highly charged microorganisms were lost in the tubing and other inner surfaces of the system. After reducing or eliminating every conceivable loss surface, the device worked efficiently.

The article shows the electric charge distributions measured on *Pseudomonas fluorescens* bacteria over a wide range of electric charges. The *Pseudomonas fluorescens* bacteria carry up to 13,000 positive or negative charges, even when there is no induction charging during their dispersal. The net electric charge on these bacteria is negative when there is no induction charging.

When the bacteria are dispersed in a highly negative induction field, the net charge on the bacteria becomes more negative and the number of highly negatively charged bacteria increases significantly. However, the percentage of bacteria with low charges remains about the same. When the bacteria are dispersed in a positive induction field, the net

charge on the bacteria becomes positive. To our knowledge, this is the first time that electric charge distributions have been measured on airborne microorganisms.

Third Article: Mainelis G, Willeke K, Baron PA, Grinshpun SA, Reponen T: Electrical Charges on Airborne Microorganisms, J. of Aerosol Science, accepted for publication, 2001.

In this article, we focus on the electric charge distributions of different particles of biological and non-biological origin. We show again the wide electric charge distribution on *Pseudomonas fluorescens* bacteria when no induction voltage is applied during their dispersal. When NaCl particles of the same size are dispersed in the same way, only very low particle charges are measured. We attribute this striking difference to the presence of electric charges in the cell walls of the bacteria. When NaCl particles are dispersed in a highly negative induction field, they become more highly charged than the bacteria dispersed in the same way. This again indicates that the cell walls of the microorganisms have their own mechanisms of regulating their electric charges. *Pseudomonas fluorescens* bacteria are gram-negative with a rather thin cell wall. Experiments with spores of gram-positive bacteria, such as *Bacillus subtilis* var *niger*, also showed a wide electric charge distribution when dispersed without induction charging.

Fourth Article: Mainelis G, Gorny R, Willeke K, Reponen T, Grinshpun SA, Yadav J, Baron PA: Effect of Electrical Charges and Fields on Viability and Injury of Airborne Bacteria, Biotechnology and Bioengineering, submitted, 2001.

In this article, we present data on the viability, metabolic injury and structural injury of charged and uncharged airborne microorganisms. In several sets of experiments, each lasting about four hours, we dispersed the microorganisms in the manner already described, passed them through the aerosol electric charge classifier and then sampled the bacteria carrying a pre-selected charge range with a BioSampler. The figures show the viability of bacteria at the beginning ($t = 0$) and at the end of the experiments ($t = 4$ hours) when there was no electric field applied across the plates of the classifier. When the induction voltage was set to zero volts during aerosol dispersion, the viability for the bacteria was found to be on the order of 50 to 60%. When a high negative charge was imposed during microbial dispersion in a highly negative induction field, the viability levels were of the same order of magnitude. However, when a high positive induction voltage was applied during dispersion, imparting a net positive charge to the bacteria, the viability was much reduced. The metabolic and structural injury levels were about the same, irrespective of the charging levels.

After the experiment at time = 0, different voltages were applied to the aerosol charge classifier and microorganisms of specific charge levels were extracted from the classifier. The negatively charged bacteria had viability levels that are normal for these bacteria. The viability of the positively charged bacteria, however, was much lower. We interpret this as follows: The bacteria naturally contain negative charges in their cell walls. When these bacteria carry positive charges, the bacterial balance is upset and the viability is reduced.

The new electrostatic sampler essentially follows the design detailed in the original grant proposal. The microorganisms are collected into a liquid which gravitationally flows down a collection surface at ground potential relative to a 4kV plate above it. The flowrate at which the bioaerosols are sampled into the device is 12.5 Lpm. The pressure drop between the inlet and the outlet of the sampler is minimal, on the order of 1 inch w.g.

Satisfaction of Specific Aims of Original Grant

All of the Specific Aims of the grant have been met:

Year 1: Development of a laboratory test system. This is described in the first two articles that are listed and discussed above.

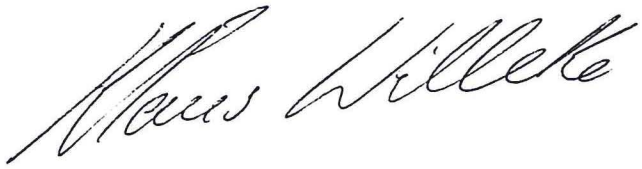
Experiments with electrically charged microorganisms. All four papers satisfy this aim.

Experiments on the charging of microorganisms and inert test particles. The first three papers satisfy this aim.

Years 2 & 3: Full-scale laboratory study of electrostatic sampling. The second, third and fourth paper satisfy this aim.

Year 3: Development of an electrostatic bioaerosol sampler and field testing. A new electrostatic bioaerosol sampler has been developed and tested in the laboratory and field. Further field-testing under conditions not anticipated in the original grant application will be performed during the two years of grant continuation. The fourth article satisfies this aim.

Note: No human subjects were tested during the grant period.



Final Invention Statement

NIOSH Grant 5 R01 OH003463-03, Years 1 through 3
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No inventions related to this grant were conceived.

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June 26, 2001

Dr. Roy M. Fleming
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Re: Final Performance Report and Final Invention Statement for NIOSH Grant 5 R01 OH003463-03 "Electrostatic Sampling of Airborne Microorganisms", 9/30/97-3/31/01

Dear Dr. Fleming,

Please find enclosed the original and two copies of the Final Performance Report and the Final Invention Statement for the above-quoted grant. The enclosed disk contains the same information. I am also enclosing three copies of the publications that are listed and highlighted in the final report.

I am very happy that the grant has been renewed for another two years. Thank you for your help. We are enjoying are continued effort.

Sincerely,

Klaus Willeke

Klaus Willeke, Ph.D.
Professor

cc: Dept. Administrative Office
Co-Investigators