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AERODYNAMIC PARTICLE SIZING OF AIRBORNE BACTERIA

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Common methods for sampling airborne microorganisms employ either collection onto a filter, impaction onto an agar surface, or impingement into a liquid. In reporting the data obtained with any of the commercially available instruments, the sampling device needs to be specified, as each device covers a different particle size range and has different physical and biological collection efficiencies. Therefore, each device needs to be calibrated for the airborne microorganisms it collects. This study was undertaken to find a suitable method for dynamic size spectrometry of airborne microorganisms, which can be applied for calibrating bioaerosol samplers.

To develop such a method, the Aerosizer, a relatively new aerodynamic particle size spectrometer (Amherst Process Instruments Inc., Hadley, MA), was examined with respect to its suitability to perform reliable dynamic measurements of airborne microorganisms over a wide particle size range. The principle of the Aerosizer is based on the acceleration of particles in a sonic expansion flow: small particles are aerodynamically accelerated to greater velocities than large particles. The particle velocity, measured by two laser beams, is thus an indicator of the particle's aerodynamic size. As the instrument's lower size limit was found to be about 0.5 µm and the upper limit is claimed by the manufacturer to be 200 µm, it appears to cover the size ranges of single-cell bacteria, fungi and pollen. Prior to using the Aerosizer with microorganisms, the instrument's factory calibration was checked with monodisperse PSL spheres ranging from about 0.3 to 3.0 µm in size. A Collison nebulizer was used to nebulize a suspension of cultured and washed microorganisms or of PSL particles. Dilution of the aerosol from the nebulizer with dry air avoided agglomeration of the droplets and evaporated the water content. The test aerosol passed through an electrical charge neutralizer and then entered an open cup from which aerosol was withdrawn at a sample flow rate of 5 L/min. An LAS-X laser aerosol spectrometer, measuring over an optical particle diameter range of 0.1 to 3.0 µm, was used in parallel with the Aerosizer to give information on the residue particles resulting from the residual nutrients, salt and bacterial slime in dried droplets not containing bacteria. Some of the sampled airborne microorganisms were also collected onto a filter for comparison of the dynamic size-spectrometric measurements with conventional enumerations from a 0.1 µm pore size polycarbonate filter.

As bacteria are accelerated in the near sonic expansion flow in the Aerosizer (Cheng *et al.*, 1993a), the particle shape and density may affect the accuracy of its aerodynamic diameter measurement, as has also been noted for the APS aerodynamic size spectrometer (Baron, 1986; Brockmann and Rader, 1990; Cheng *et al.*, 1993b). The Aerosizer's response to non-spherical bacteria was investigated by using the sixth stage of a six-stage Marple Cascade Impactor with a 1.0 mm nozzle diameter for inertial calibration of the two dynamic instruments. *Pseudomonas fluorescens* (ATCC 13525) was selected as our major model bacterium. As many as six washings were performed with this organism to reduce the amount of residue in the bacterial suspension prior to nebulization. The number of washings was found to affect the bacterial size. The reported size of *P. fluorescens* varies among authors, ranging from 0.3 to 0.8 µm in diameter and from 1.0 to 3.0 µm in length. We used Scanning Electron Microscopy to examine the shape and size of *P. fluorescens* cells used in our experiments. The micrographs showed that we generated rod-shaped cells of 0.4 to 0.7 µm in width and 1.5 to 2.0 µm in length. Several other bacteria of relatively small size were aerosolized from suspensions that had been washed three times: *Bacillus alcalophilus* (ATCC 21522) of 0.7-0.9 µm width and 3-4 µm length, *Bacillus megatherium* (ATCC 14581) of 1.2-1.5 µm width and 2-5 µm length, and a coccus, *Streptococcus salivarius* (ATCC 13419) with a diameter of 0.8-1.0 µm (exists in irregular chains).

We have found that the physical size of the aerosolized bacteria notably decreases after the first washing and a little more after additional washings. This suggests that a significant amount of nutrients and bacterial slime coat the bacteria, if not washed. The procedure and degree of bacterial washing should, therefore, be specified for calibration purposes. As residue material is removed from the bacteria, their shape may change and, thus, the width of the measured size distribution. At this time, it is not clear whether elongated particles are randomly distributed in the sensing zone of the Aerosizer, as was observed with the functionally similar APS aerodynamic size spectrometer (Brockmann and Rader, 1990; Cheng *et al*, 1993b). The particle size distribution was found to narrow, as its peak shifts to a smaller size with increased washings. Such a narrowing may also occur upon approaching the lower limit of detection.

The Aerosizer-measured bacterial concentration was found to decrease linearly with increasing dilution of the aerosol flow, when a diluter with a dilution filter is inserted upstream of the Aerosizer. This tendency was observed even for bacteria below 0.5 µm. While the Aerosizer's absolute counting efficiency decreases below 0.5 µm, this result shows that, in principle, it can be used for relative concentration measurements down to 0.3 µm.

We have concluded that dynamic particle sizing of airborne bacteria can be successfully performed with the Aerosizer. This instrument is ideally suited for calibrating or evaluating bioaerosol samplers in the laboratory, in preparation for their use in the field.

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