

# Utilization of bursting bubbles for aerosolization of biological and abiological aerosols

Gediminas Mainelis<sup>1\*</sup>, David Berry<sup>1</sup>, Hey Reoun An<sup>1</sup>, Maosheng Yao<sup>1</sup>, Kevin DeVoe<sup>2</sup>, and Rudolph Jaeger<sup>3</sup>

<sup>1</sup>Rutgers University, Dept. Env. Sciences, 14 College Farm Rd., New Brunswick, NJ 08901, USA;

<sup>2</sup>BGI Inc., 58 Guinan Street, Waltham, MA 02451, USA;

<sup>3</sup>CH Technologies, Inc., 263 Center Avenue, Westwood, NJ 07675, USA.

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Evaluation of pathogen collection methods, inhalation studies, instrument calibration, and other aerosol investigations require stable and reliable aerosol and bioaerosol generators. Commercially available devices, such as the Collison nebulizer, are able to produce high concentrations of aerosol; however in such particle generators, the suspension is "recycled" internally and the bacteria are repeatedly subjected to shear force stress leading to injury and fragmentation of microorganisms, especially in sensitive strains. Ulevicius et al. (1997) proposed a generator in which particles, suspended in a liquid, are produced from a bubbling liquid. Dry air injected tangentially reduces the moisture content of the airborne droplets and carries the particles away from the bubbling source and out of the device. This generator was shown to effectively aerosolize polystyrene latex (PSL) particles of 0.71-5.1  $\mu\text{m}$  in size. However, even in this generator a certain fraction of the suspension was still being recycled and the particle output depended on the distance between the drying jets and the liquid surface which varied as aerosolization progressed.

In an effort to improve aerosolization methods for microorganisms, especially of sensitive species, we designed and analyzed a new particle generator that utilizes a bursting bubble principle and eliminates carrier fluid reuse. Utilization of bursting bubble design along with elimination of carrier fluid reuse was expected to minimize shear forces on particles being aerosolized. In this Liquid Sparging Aerosolizer (LSA), a suspension of particles or microorganisms is pumped at a flow rate of 0.2 to 2 mL/min to the top surface of a porous stainless steel disk (frit) where it forms a thin film. Air is then forced through the disk into the film (sparging) causing it to break into bubbles that subsequently burst, releasing particles into the air. The released particles are then captured by the sparging air stream and are carried away. A fraction of particles and liquid not captured by the air stream (our experiments showed that this fraction is small) are collected at the bottom of the glass vessel and play no further role in the aerosolization process. Thus, sensitive particles, such as microorganisms, participate in the generation process only once and there are no strong shear forces involved. The dimensions of the glass vessel are the same as those of a common Collison nebulizer (BGI Inc., Waltham, MA, USA).

We tested performance of the LSA with disks of different pore sizes (0.2, 0.5, 2.0 and 10.0  $\mu\text{m}$ ) and different air flows (2 - 30 L/min) through the porous disks while generating polydisperse and monodisperse particles. Our tests showed that the use of 0.5 and 2.0  $\mu\text{m}$  porosity disks resulted in the highest output of PSL particles in the desired size range, i.e., comparable to bacterial size. Each pore size seemed to have an optimal air flow rate; the resulting aerosol concentration increased with increasing suspension delivery rate. When an LSA fitted with 2  $\mu\text{m}$  disk and a Collison nebulizer were used to aerosolize a Polystyrene Latex (PSL, 5.1  $\mu\text{m}$ ) suspension at equal concentration (air flow rate of 10 L/min), the LSA produced a greater particle concentration compared to Collison nebulizer.

The LSA also demonstrated stability of output concentration when continuously aerosolizing for 180 min. More importantly, the size distribution of injury-sensitive *Pseudomonas fluorescens* bacteria remained virtually unchanged during 90 minutes of continuous aerosolization with LSA. In fact, there was no (0%) viability loss, whereas the bacterial spectrum produced by a Collison nebulizer changed significantly over 90 minutes, leading to a 50% loss in viability. Similar preservation of culturability was observed in experiments with vegetative cells of *Escherichia coli* and *Micrococcus luteus* bacteria.

In our newest development, the LSA was fitted with an enlarged (90 mm) sparging surface featuring pore size of 2  $\mu\text{m}$ . This large unit is designed to operate at air flows of up to 100 L/min, a flow rate making this unit advantageous in large scale releases of biological and abiological particles.

The obtained results indicate that the new instrument utilizing bursting bubbles could be successfully used in various applications where extended delivery of stable and undamaged biological aerosols and select agents are required.

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