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Bacterial aerosol neutralization by aerodynamic shocks using an impactor system: Experimental results for *E. coli* and analysis

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ABSTRACT

Neutralization of bacterial aerosol releases is critical in countering bioterrorism. As a possible bacterial aerosol neutralization method that avoids the use of chemicals, we investigate the mechanical instabilities of the bacterium cell envelope in air as bacteria are passed through aerodynamic shocks. To carry out this fundamental investigation, an experimental impactor system is developed to collect the bacteria after they pass through a controlled shock, and a detailed computational study is carried out to determine the impactor operating conditions that lead to bacterial break-up. Specifically, the bacteria experience relative deceleration because of sharp velocity changes in the aerodynamic shock created in the experimental impactor system. Computational model results indicate that vegetative *Escherichia coli* cells require a critical acceleration of $3.0 \times 10^8\ m/s^2$ to break-up. As predicted in computations, experimental findings demonstrate that aerosolized *E. coli* cells that pass through aerodynamic shocks created in the experimental impactor system are an order of magnitude less likely to retain viability than those that pass through the impactor at conditions which do not lead to the generation of an aerodynamic shock, and therefore, do not reach the critical acceleration required for break-up.

1. Introduction

Anthropogenic bacterial aerosols can be used as bioterrorism agents and contribute to the background environmental aerosol. In urban outdoor environments background levels reach 850 cfu/mL (Bovallius et al., 1978; Di Giorgio et al., 1996; Lighthart and Shaffer, 1997; Mancinelli and Shulls, 1978), where cfu refers to colony forming units. Indoor environments contain less bioaerosol. The suspended bacterial particles (both background and anthropogenic) usually range in size from 1 μm (single cells) to 10 μm (multiple cells or cells associated with debris) (Vitko, 2005). In order to effectively mitigate the threat of a biological agent release, effective methods for both sensing and neutralization are necessary.

With respect to sensing, different approaches for biological aerosols have been studied and can be classified into four categories: (1) nucleic acid-based, (2) structure-based, (3) chemistry-based and (4) function-based (Vitko, 2005). Assuming the detection of these agents is possible, current methods are not effective in neutralizing the aerosol cloud at its source and mostly include containment of the aerosol release in order to minimize human exposure. These methods are limited to indoor environments and employ techniques such as high efficiency particulate air (HEPA) filtration, electrostatic precipitation, steam condensation, ultraviolet (UV) inactivation as well as diverting airflows (Vitko, 2005).

The use of aerodynamic shocks holds promise as an alternative way to neutralize bacterial aerosols. The shock can be applied at the point of release both in indoor and outdoor air with the advantage of avoiding chemicals. The aerodynamic shock represents a sharp change in fluid properties (velocity, temperature, pressure). Due to its inertia, a bacterial particle suspended in air will not be able to immediately adjust to a sharp drop in gas velocity and will therefore experience a relative deceleration. Rayleigh–Taylor instabilities in the bacterial membrane arise if the bacterial particle is accelerated (or decelerated) in a perpendicular direction to the bacteria–gas interface (Chandrasekhar, 1961; Joseph et al., 1999). In previous computational work, we have computed the critical accelerations and Weber numbers needed for several bacterial aerosols to break-up (Sislian et al., 2009). Higher surface tension and smaller diameter of the bacterium increase the critical accelerations needed for break-up (Sislian et al., 2009). An impactor system, shown in Fig. 1, has been built and modeled to achieve different bacterial accelerations through a well-defined aerodynamic shock

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(Sislian et al., 2009). After passing through the shock, the bacterial aerosol is collected at low velocity ($<10\,m/s$) to avoid break-up at the point of impaction (Sislian et al., 2009).

A computational model describing the evolution of both gas and particle velocity and temperature was developed for the current impactor system (see Fig. 1) (Sislian et al., 2009). The model calculates accelerations of bacterial particles of up to $10^{10}\,m/s^2$ in the current impactor system. These accelerations are sufficient to break-up gram-negative vegetative *E. coli* cells. However, for example, gram-positive spores of *B. atropheus* which have a higher surface tension and smaller diameter will not reach the critical acceleration required for break-up in the current system (Sislian et al., 2009). Furthermore, to validate the model, the computationally calculated stagnation pressures for different operating conditions were compared with experimental Pitot pressure measurements (Sislian et al., 2009). The calculated and measured data match closely (Sislian et al., 2009).

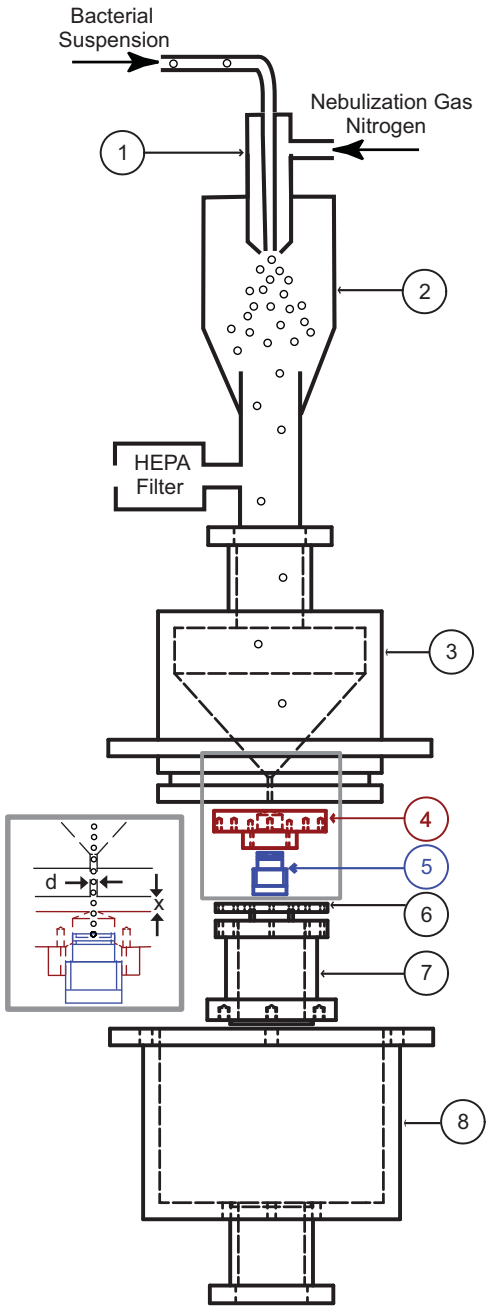

Fig. 1. Schematic of the experimental system. (1) Meinhard nebulizer with a concentric nozzle mixing a liquid bacterial suspension feed and a dispersion gas (N_2), (2) nebulization chamber to collect large droplets, (3) converging nozzle with an exit diameter of $d=0.5\,mm$, (4) and (5) a flat surface with a 0.5 mm hole combined with a screw that accommodates the collection substrate make up the deceleration tube, (6) 1.4 mm spacer fixes the spacing x between the impactor and nozzle to 0.6 mm, (7) support holding the deceleration tube and flat surface at fixed distance from the nozzle and (8) exit chamber.

However, the computational model predictions of bacterial neutralization have not been confirmed previously. Some experimental data exist in the literature (Horneck et al., 2001; Lundbeck and Skoldber, 1963; Teshima et al., 1995) on the use of shock waves to neutralize bacteria; however, these studies are limited to bacterial liquid suspensions or powders and do not assess the effects of a shock in the aerosol phase.

The present work focuses on assessing the effect of the aerodynamic shock on *E. coli* cells, which are predicted by our computational model to break-up with our current impactor system. The viability of the *E. coli* is determined after the aerosolized cells pass through the impactor at different operating conditions and are collected. As predicted in computations, experimental findings demonstrate that aerosolized *E. coli* cells that pass through an aerodynamic shock created in the experimental impactor system are an order of magnitude less likely to retain viability than those that pass the impactor at conditions which do not lead to the generation of an aerodynamic shock, and therefore, do not reach the critical accelerations required for break-up.

2. Experimental materials and methods

2.1. Experimental setup

The impactor system discussed in our previous study (Sislian et al., 2009) was used in the experimental setup and is shown in Fig. 1. The bacterial suspension (see Section 2.2) is aerosolized using a capillary nebulizer (TR-30-A1, Meinhard Glass Products) at a gas (N_2) critical flow rate of 0.2 L/min (see Fig. 1; parts 1-2). The impactor (see Fig. 1; parts 3–8) operates at a critical flow rate of 2.3 L/min at shock conditions. As shown in Fig. 1, a high efficiency particulate air (HEPA) filter is installed between the nebulizer (part 2) and the impactor system (part 3) to allow particle-free air to compensate for the higher impactor flow rate, thereby maintaining the upstream impactor pressure at 1 atm.

2.1.1. Nebulization

The phosphate buffered saline (PBS) (BP2438-4, Fisher Scientific) suspension containing the *E. coli* cells (see Section 2.2) is fed via a syringe pump (KDS410, Kd Scientific) at a liquid flow rate $Q=0.05\,mL/min$ for 20 min. The liquid is fed at a specified bacterial concentration (see Section 2.2) determined by the optical density measurement at 600 nm (OD_{600}). Nitrogen is supplied to the nebulizer at a pressure P_n via a pressurized tank. When the liquid is mixed with the air in the annular region of the nebulizer tip, droplets containing bacterial cells are formed. The bacterial aerosol concentration and droplet size can be adjusted by changing: Q, OD_{600} and P_n. Increasing Q and decreasing P_n increases the average droplet diameter (and volume). Increasing OD_{600} increases the number of cells per volume of liquid nebulized. Collectively, increasing Q and OD_{600} and decreasing P_n increases the number of cells per droplet. With the exception of P_n, Q and OD_{600} also increase the number of cells input into the system in a given time. In all the experiments, the nebulizer was operated at $Q=0.05\,mL/min$, $OD_{600}=0.1$, and $P_n=2\,atm$. The cell concentration at $OD_{600}=0.1$, as measured using fluorescence cytometry (see Section 2.3), was $2.66\pm0.28\times10^7$ cells/mL. The