

Collection of airborne spores from combustion environments

S.A. Grinshpun¹, A. Adhikari¹, C. Li¹, T. Reponen¹, M. Schoenitz², E.L. Dreizin², M. Trunov³

¹ Center for Health-Related Aerosol Studies, University of Cincinnati, Cincinnati, OH 45267, USA

² Department of Chemical, Biological and Pharmaceutical Engineering, NJIT, Newark, NJ 07102, USA

³ Reactive Metals, Inc., Newark, NJ 07103, USA

Keywords: aerosolization, bacteria, spore, combustion, temperature, filter collection

Destruction of biological agents is the subject of increasingly active research and development. Energetic materials are currently being developed with the added capability to effectively inactivate stress-resistant microorganisms. This is of particular interest, as in a military/counterterrorism situation biological agents may become aerosolized and should be neutralized rapidly, before larger areas become contaminated. Consequently, there is a need in developing and validating adequate methods and protocols for testing the biocidal effectiveness of such energetic materials. We have recently designed and built a state-of-the-art experimental facility for assessing the survival of aerosolized microorganisms exposed to the combustion of various materials. The facility includes an aerosolization chamber, a combustion chamber, and a system for measuring the physical (through real-time monitoring) and biological (through collection and viability analysis) characteristics of bioaerosol particles. Particles passing through the chambers are collected on a filter, which is continuously exposed to combustion products during the entire test. There is currently no data on microbial inactivation occurring on the collection filter due to this exposure. This study addresses this knowledge gap.

A challenge bioaerosol of *Bacillus subtilis* endospores, generated from suspension with a six-jet Collison nebulizer was mixed with HEPA-filtered dry air. Charge-equilibrated by passing through a 10 mCi Kr⁸⁵ neutralizer (TSI, Inc., USA), the bioaerosol was measured with an optical size spectrometer (Grimm Technologies, Inc., Germany). Following a 10-min nebulization period, a stable concentration was achieved in the range of 10^2 – 10^3 spores/cm³. The spores were collected on five polycarbonate filters with no burning in the combustion chamber. After deposition on the filters, two pre-loaded filters served as controls, while three others were exposed to combustion products as a strand (diameter=1/2", length=10") burned in the combustion chamber for up to 2 min. The strand consisted of an energetic Al-MoO₃ nanocomposite and paraffin wax as a binder. Combustion products were aluminum oxide, molybdenum oxide, water, carbon oxides and soot. Estimates derived from continuous monitoring of the chamber wall temperature suggest that the air inside the chamber was heated well in excess of 200°C. However, gaseous combustion products were cooled

down prior to reaching the filters so that the pre-loaded spores were not exposed to high temperatures. Filter collection from the combustion environment was conducted at time intervals ranging from 20 to 80 s. *B. subtilis* spores were extracted from the filters using a standard extraction method and cultivated on TSA agar. After incubation for 18 h, the surviving spore fraction was calculated based on the bacterial colony forming units in control and treated samples.

The microbial inactivation occurring on the collection filters was found to depend on the time for which the filter was exposed to combustion products. The data suggest that the surviving fraction of spores decreases exponentially with time (see Figure). It is seen that a significant fraction of microorganisms that were viable when collected on the filter, may lose viability as a result of the exposure to the combustion products. Inactivation of more than 50% of initially viable spores was observed for collection times as short as 20 s.

The data suggest that distinguishing the biological inactivation in the aerosol from that occurring on the collection filter poses a considerable challenge. To minimize the detrimental effect of the combustion products on the viability of spores collected on a filter, the collection time should not exceed a few seconds. This is impractical in many cases, which in turn raises a question of the suitability of filter collection for these experimental conditions. Samplers with liquid collection medium, such as impingers and wet cyclones, are currently being examined as possible alternatives.

The study was funded by the US Department of Defense (grant HDTRA-1-08-1-0012).

