University of Colorado at Boulder
College of Engineering and Applied Science

Department of Civil, Environmental and Architectural Engineering
1111 Engineering Drive, Room #441
Campus Box: UCB 428
Boulder, CO 80309-0428

Telephone: 303.492.7211
Facsimile: 303.492. 7317
http://stripe.colorado.edu/~hernando
email:mark.hernandez@colorado.edu

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Via Electronic Mail
No Hardcopy to Follow

FROM: MARK HERNANDEZ, Principal Investigator

RE: RESULTS SUMMARY FOR DYNAMIC PARTITIONING POTENTIAL OF MONODISPURSED AEROSOLIZED PURE CULTURE *E. coli* INTO A PROTOTYPE CYLINDRICAL PRESSURIZED WATER FEATURE UNDER DEFINED ENVIRONMENTAL CONDITIONS (25C @ 40% RH)

A time series of experiments was performed by aerosolizing known quantities of stationary phase *E. coli* cells into a well characterized bioaerosol chamber (Peccia et al, 2001). The chamber hosted a grounded, fan-pressurized cylindrical prototype water feature with a reservoir volume of approximately 2L; recycling this reservoir volume across a continuously wetted stainless steel perforated metal cylinder with approximately 2490 cm^2 of wetted area, in contact with aerosol. Airborne bacterial cells were generated to a concentration > 10^7 m^{-3} over a period of 15 minutes, after which the water feature operation was initiated, and allowed to run in this chamber atmosphere for 12 hours (overnight). The *E. coli* content of the water feature reservoir was quantified prior to and following its operation, facilitating the determination of an overall bioaerosol flux rate from the chamber air into the operating feature.

A wild type *E. coli* strain presenting an F pilus, was chosen as the candidate for this study, because pure cultures of this bacterium have been used as a model for the environmental behavior of gram negative bacteria in many military and scientific studies. The *E. coli* variant used here provided for the unambiguous and quantitative tracking of these microbes partitioning from an aerosol phase into an aqueous phase. The tenant defining this approach is as follows: having started these investigations with a clean feature containing sterile water, any *E. coli* present in the reservoir must partition into the water circulating on the screen of the feature. Where executed in a timed series, the flux of microbes into the feature can thus be estimated. Results from challenges with these cells can conservatively approximate partitioning potential of gram negative bacterial bioaerosols into this feature configuration. The preparation and contained aerosolization of bacterial cells is a laborious process, and a detailed protocol is referenced in a separate, peer-reviewed document (Peccia, et. al, 2001).

EXPERIMENTAL DESIGN: A stock suspension of pure *E. coli* cells (American Type Culture Collection # ATCC 700926) was raised to stationary growth. Immediately following liquid transfer, cells were introduced to a $1m^3$ bioaerosol chamber using ultra-pure carbon dioxide (20 psi) through a six-jet Collison nebulizer for 15 min, at 24 ^oC, and 40% RH. Cells retained in the water feature's reservoir were counted directly under 1100x magnification using Nikon Eclipse E400 epifluorescence microscope. The quantity of cells was estimated using widely accepted methods (Hernandez et al, 1999 and Henningson et al 1997).

RESULTS: The bioaerosol chamber was loaded with greater than $10^8/m^3$ stationary phase airborne *E. coli*, a concentration which decayed several orders of magnitude overnight; this was a level great enough to support observations for partitioning analyses.

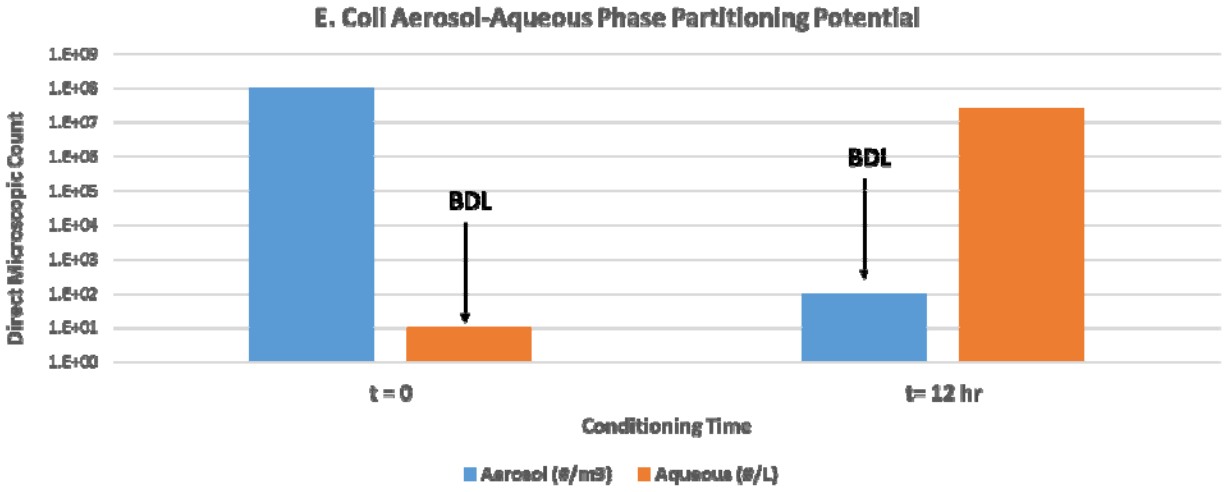

Figure 1. Direct microscopic counts of *E. coli* cells in chamber air (■) at 24 ºC and 40% RH, and in retained in stainless steel water feature reservoir (■). Bar height represents averaged count per media volume (m^3 for air, or L for water).

DISCUSSION: Exposure to bioaerosol under these chamber conditions caused a significant increase the concentration of *E. coli* cells in the reservoir of this stainless steel water feature. As judged by direct microscopic count, bioaerosol chamber exposure increased the total cell numbers in the water feature by more than 10^6/L when challenged with an aerosol initially containing more than 10^8 *E. coli* cells over the period of 12 hours. When controlled for aging effects, aerosol hosting stationary phase *E. coli* cells appeared to partition into the operating water feature at a gross rate of c.a. 300 cm^{-2} hr^{-1}.

Henningson, E. W., M. Lundquist, E. Larsson, G. Sandstrom, and M. Forsman, 1997, A comparative study of different methods to determine the total number and the survival ratio of bacteria in aerobiological samples.: Journal Aerosol Science, v. 28, p. 459

Hernandez, M., S. L. Miller, D. W. Landfear, and J. M. Macher, 1999, A Conmbined Fluorochrome Method for Quantitation of Metabolically Active and Inactive Airborne Bacteria: Aerosol Science and Technology, v. 30, p. 145.

Peccia, J., Werth, H., Miller, S. L., and Hernandez., M. 2001 The Effect of Relative Humidity on the UV-induced Induced Inactivation of Airborne Bacteria. *Aerosol Science and Technology* v. 35 p. 728-740.