

ESTIMATING SOLAR AND NONSOLAR INACTIVATION RATES OF AIRBORNE BACTERIA

Tania Paez-Rubio*

Arizona State University.

Jordan Peccia

Arizona State University.

Author address: Arizona State University. Engineering Center G-wing. Room 252. Tempe. 85287-5306. Phone number: 001-480-965-3296. Fax number: 001-480-965-0557. e-mail: tania.paez@asu.edu

ABSTRACT

Land application of biosolids is a wide spread practice in the US, Canada and Western Europe. Fundamental information to characterize the relationship between atmospheric conditions, source concentration, transport, and airborne fate of bioaerosols emitted during the application of biosolids is needed to assess exposure to workers and the public. Pilotscale bioaerosol reactor experiments that independently measure the solar and nonsolar (absence of solar radiation) inactivation rates of airborne *Mycobacterium parafortuitum* and *Escherichia coli* were performed. Direct fluorescent microscopy measurements for total airborne bacteria and culture-based assays were used to measure concentrations in a one m³ reactor that was transparent to UV-A and UV-B wavelengths and to produce decay curves of airborne bacteria under moderate and high ranges of relative humidity (RH). Relative humidity strongly influenced airborne inactivation rates in both bacteria. In addition, *E. coli* was more susceptible to solar and nonsolar airborne decay than *M. parafortuitum*. Results demonstrate, at moderate RH, that nonsolar mechanisms dominate the inactivation of airborne *E. coli* while solar mechanisms dominate *M. parafortuitum* inactivation.

Keywords: transport, bioaerosols, solar inactivation, relative humidity.

INTRODUCTION

Aerosolization and subsequent pathogen transport caused by wastewater reuse or biosolids application onto agricultural fields may result in exposure of workers and nearby residence to airborne wastewater derived microorganism—especially where suburban development areas are encroaching on agricultural land. An understanding of the fate of bacterial bioaerosols is necessary for predicting the transport of viable microorganisms to off site locations and assessing exposure to workers and the public (Dowd et al., 2000). Dispersion models have previously been used to estimate downwind bioaerosol concentrations from aerosol sources as a function of atmospheric conditions, source generation rates, and bioaerosol inactivation rate coefficients (Lighthart and Mohr, 1987,

Dowd et al., 2000). This approach requires inactivation rate coefficients for both, solar and nonsolar decay.

Bioaerosol solar inactivation rate coefficients determined in controlled studies have not been reported in the literature and a paucity of qualitative information exists regarding the response of airborne bacteria to solar radiation. Because parameters that affect dispersion, such as atmospheric stability, mixing height, wind speed, are often different between periods of day and night, and bioaerosol source emission flux may be variable, field studies (Teltsch et al., 1978, Teltsch and Katzenelson, 1980, Bausum et al. 1983, Lighthart and Shaffer, 1995) that measured diurnal concentrations of culturable bioaerosols have led to inconsistent conclusions regarding whether solar radiation results in lower airborne concentrations. Hence, the estimation of solar inactivation rates from these types of full-scale studies is problematic. Moreover, previous studies on the nonsolar inactivation of airborne bacteria have identified relative humidity as an important environmental factor influencing these inactivation rates (Lighthart and Mohr, 1987, Cox 1995).

The objective of this work was to estimate solar and nonsolar inactivation rates of two physiologically different bacteria when airborne at mid (50-60%) and high (85-95%) relative humidity levels, and in liquid suspension.

METHODOLOGY AND DEVELOPED STAGES

Two pure bacterial cultures, *Escherichia coli* (ATCC, #15597) a facultative gram-negative, non-spore-forming, bacillus and *Mycobacteria parafortuitum* (ATCC, #19689), a rapid growing, acid-fast bacillus, were used for pilot-scale inactivation experiments. *E. coli* is an accepted indicator of fecal contamination and *M. parafortuitum* is a previously used surrogate for ultraviolet inactivation of *Mycobacteria tuberculosis*. Both culturable and total bacteria were quantified in the aerosol and liquid suspension experiments. For culturable bacteria, the standard plate count method was used (APHA, 1995).

Epi-fluorescent microscopy was used to enumerate total bacteria (culturable and nonculturable). An aerosol batch reactor was built using 5 mil thick Aclar Fluoropolymer film, 22C. The polymer film was attached to a 1 m³ cubic aluminum frame (Fig.1). An air-jet nebulizer operated at 20 psi was used to aerosolize pure bacterial cultures into the reactor. Air was supplied by a ¼ horsepower pump and filtered through a mixture of anhydrous calcium sulfate and activated carbon to remove water and organics vapor from the pump. Cells were collected in glass swirl liquid impingers that contained 15 ml of sterile phosphate buffered saline solution (PBS, 30mM phosphate buffer, pH 7.2 and 125mM NaCl). Airborne cells were sampled through Teflon® tubes, that extended from the impinger (outside the reactor) into the center of the reactor. Impingers were connected to a vacuum pump, ¼ HP that operated at a pressure greater than 15 mmHg to obtain a flow rate of 12.5 ± 0.5 L/min.

For liquid suspension experiments, cell solutions in 50 ml of PBS were placed in an uncovered standard petri dish. The solution was mixed on a magnetic stir plate at approximately 200 rpm with a Teflon coated stir bar. The top of the petri dish was covered with Aclar Fluoropolymer film, 22C.

All reactor experiments yielded total and culturable cell concentrations as a function of time. First order rate approximations were used to estimate the decay of (1) total bacteria

and (2) culturable bacteria in the presence and absent of solar radiation according to the following relationships:

(1) (2)

where N_t [#cells/m³] is the total concentration of bacteria. The coefficient for the loss rate of total bacteria is due to deposition on the reactor wall and other physical removal processes and is termed $k_{deposition}$ [h⁻¹]. The value N_c [#CFU/m³] is the concentration of culturable bacteria.

t deposition N k d t d N = () c n o n s o l a r s o l a r d e p o s i t i o n c d N k k k N d t = - + **carbon filter/1m² desiccator vacuum pump Sample collection by liquid impinger aerosol nebulizer (Cell solution) 1 ml mair pump thermo-hygrometers humidifier UV-A and UV-B radiometer**

RESULTS

Table 1 presents first order rate coefficients, k_{solar} (h⁻¹) and $k_{nonsolar}$ (h⁻¹) for both microorganisms at mid and high RH levels, and liquid suspension. The solar inactivation rate coefficient was greater than nonsolar rate only in *M. parafortuitum* at moderate RH. In *E. coli*, nonsolar inactivation was the dominant mechanism at moderate RH levels. At high RH, solar and nonsolar inactivation rates were not statistically different (paired t-test, $\alpha=0.05$) in both microorganisms. These solar rate coefficients were derived at peak solar intensities and correspond to a maximum solar rate coefficient. Rate coefficients (k) normalized to the irradiance (I) (Table 1) derived in k'_{solar} (cm² W⁻¹ h⁻¹). This normalization is necessary to compare inactivation rate coefficients measured at slightly different UV-A and UV-B intensities for experiments presented here and in the literature. Statistically (paired t-test, $\alpha=0.05$), no difference in k'_{solar} for *E. coli* at 95% and 60% RH levels was observed whereas, *M. parafortuitum* solar inactivation decreased with increasing RH levels. At 95% RH, k'_{solar} for *E. coli* was approximately fifteen times greater than the coefficient for *M. parafortuitum*. At the moderate RH levels tested, *E. coli* and *M. parafortuitum* k'_{solar} coefficients were not different. In addition, *M. parafortuitum* rate coefficients were not different between 95% RH level and in liquid suspension ($\alpha=0.05$). Under the same conditions, the solar inactivation rate for *E. coli* at 95% RH level was markedly higher (c.a. six times) than the rate in liquid suspension.

TABLE 1: Solar (k_{solar}) and nonsolar ($k_{nonsolar}$) inactivation rate coefficients \pm standard error.

E. coli *M. parafortuitum* RH level Temperature range (°C) **3.6 ± 5.5** ($I=0.065$) **6.9 ± 0.9** ($I=0.07$) Moderate **7.8 ± 1.4** ($I=0.049$) **0.6 ± 0.9** ($I=0.07$) High $k_{solar} \pm$ std. Error (h⁻¹) **3.2 ± 0.6** ($I=0.12$) **1.9 ± 0.5** ($I=0.11$) Liq. suspension 22 - 36 **20.1 ± 5.2** **2.8 ± 0.3** Moderate **6.7 ± 0.8** **1.3 ± 0.5** High $k_{nonsolar} \pm$ std. error (h⁻¹) **1.0 ± 0.4** **1.3 ± 0.3** Liq. suspension 17 - 23 Note: I represents the average irradiance in W/cm²

Total airborne bacterial concentrations were also determined to ensure that the measured coefficients were compared on equal bases for all independent experiments. Because the reactor is sealed, deposition was assumed to be the only physical removal mechanism and the rate coefficient ($k_{deposition}$) could be estimated from the decay of total bacterial

counts. No difference in $k_{deposition}$ values was observed in all solar and nonsolar aerosol experiments ($\ll=0.05$), demonstrating that changes in relative humidity and solar radiation did not affect the physical loss rate of cells inside the reactor.

DISCUSSION

A more fundamental understanding of the solar inactivation of bioaerosols is necessary for predicting fate and transport.

Previously, Teltsch and coworkers (1980) determined airborne inactivation rate coefficients of aerosolized, fluorescently labeled *E. coli* at a sprinkler irrigation site in Israel. The inactivation rate coefficients were 31.6 h⁻¹ in the early morning with a 95% RH level and low solar radiation, and 237 h⁻¹ in the afternoon with 50% RH level and high solar radiation. These rate coefficients accounted for both solar and nonsolar mechanisms. While results agree with those presented here in behavior, differences in experimental conditions make the comparisons of magnitudes more tenuous. In pilot scale observations, we and others (Heidelberg et al., 1997) have observed a large loss in culturability of gram negative organisms upon aerosolization, followed by a much slower decay rate. The magnitude of this initial loss in culturability was approximately 96% at high RH and 99.6% at mid RH and observed only in *E. coli*. We attributed this die off to stresses during nebulization and this effect was not included in the calculation of reported rate coefficients. The field experiments in Israel accounted for this initial die off (as is appropriate for sprinkler irrigation) and hence reported higher rates.

Researchers using germicidal irradiation (UV-C) on airborne bacteria demonstrated that the gram negative *Serratia marcescens* inactivation rate increased with decreasing relative humidity (Riley and Kaufman, 1972, Peccia et al., 2001). However, at lower UV-C irradiance tested (nearer to the equivalent UV-A and UV-B germicidal effect in sunlight), there was no difference between inactivation rates measured at the moderate and high range RH levels (Riley and Kaufman, 1972). In the experiments reported here, a RH dependency for *E. coli* was observed for nonsolar inactivation. *Mycobacteria parafortuitum* solar and nonsolar inactivation rates were a function of RH, and are minimized at high RH levels. Solar inactivation rate was ten times greater at 50% RH than in nearly saturated air (95% RH). A similar trend was reported in UV-C experiments with acid fast *M. parafortuitum* and other gram positive organisms (Peccia et al, 2001 and Xu et al, 2003). Comparisons between UV-C and solar inactivation experiments should be considered tentative, due to the differences in UV intensities and wavelengths, and differences in type of photoproduct that are produced under those conditions (Miller et al, 1999). Results from liquid suspension experiments demonstrated that rate coefficients derived in liquid underestimate solar inactivation in air. Previous research; Peccia and coworkers (2001), and Peccia and Hernandez (2004) demonstrated that when cell water sorption increased, inactivation rates decreased.

Overall, the measured response of the two airborne bacteria to environmental challenges suggested that inactivation of these microorganisms was controlled by different mechanisms at moderate RH. For *M. parafortuitum*, solar inactivation was the most significant inactivation mechanisms throughout a diurnal cycle. However, natural decay (nonsolar) mechanisms dominate *E. coli* inactivation. Relative humidity was an important environmental factor for controlling inactivation in both microorganisms. The results presented here also provide insight for choosing appropriate indicator microorganisms for

airborne contamination. The rate coefficients for *M. parafortuitum* suggest low aerosol inactivation when compared to the time needed for offsite transport—under maximum inactivation conditions (mid RH level, high solar irradiance) and a 2m/s wind velocity, the time (Lighthart and Mohr, 1987; Lighthart and Frisch, 1976) predicted for a one half kilometer transport, would result in a 50% survival. A similar scenario in *E. coli* would result in a 18% survival at one half kilometer. The higher loss rate in *E. coli* supports the use of an alternative microorganism for air, such as *Clostridium sp.*, which has been previously used as an airborne biosolid indicator (Pillai et al., 1996).

nonsolar inactivation solar inactivation total

M. parafortuitum moderate RHE. ***E. coli*** high RHE. ***E. coli*** moderate RHE. ***M. parafortuitum*** high
 H0246810120 2 4 6 8 10 12 14 16 18 20 22 24timeaverage rate (hr-1)00.511.522.50 2 4 6
 0 12 14 16 18 20 22 24timeaverage rate(hr-1)05101520250 2 4 6 8 10 12 14 16 18 20 22
 4timeaverage rates (hr-1)02468101214160 2 4 6 8 10 12 14 16 18 20 22 24timeaverage
 ates (hr-1)5

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