Environ. Sci. Technol. **2005**, *39,* 1584−1590

Bioaerosol Emission Rate and Plume Characteristics during Land Application of Liquid Class B Biosolids

BENJAMIN D. TANNER,*,† JOHN P. BROOKS,† CHARLES N. HAAS,‡ CHARLES P. GERBA,† AND IAN L. PEPPER†

Department of Microbiology and Immunology, University of Arizona, Tucson, Arizona 85721, and Department of Civil, Architectural, and Environmental Engineering, Drexel University, Philadelphia, Pennsylvania 19104

This study investigated bioaerosol emission rates and plume characteristics of bioaerosols generated during land application of liquid Class B biosolids. In addition, it compared the rate of aerosolization of coliphages and total coliform bacteria during land application of liquid Class B biosolids to the rate of aerosolization during land application of groundwater inoculated with similar concentrations of *Escherichia coli* and coliphage MS2. Air samples were taken immediately downwind of a spray applicator as it applied liquid (~8% solids) biosolids to farmland near Tucson, Arizona. Air samples were also collected immediately downwind of groundwater seeded with MS2 and *E. coli* applied to land in an identical manner. Air samples, collected with liquid impingers, were taken in horizontal and vertical alignment with respect to the passing spray applicator. Vertical and horizontal sample arrays made it possible to calculate the flux of microorganisms through a virtual plane of air samplers, located 2 m downwind of the passing spray applicator. Neither coliphages nor coliform bacteria were detected in air downwind of spray application of liquid Class B biosolids. Based on limits of detection for the methodology, the rate of aerosolization during land application of liquid biosolids was calculated to be less than 33 plaque forming units (PFU) of coliphage and 10 colony forming units (CFU) of coliform bacteria per meter traveled by the spray applicator. The rate of aerosolization during land application of seeded groundwater was found to be, on average, 2.02×10^3 CFU *E. coli* and 3.86×10^3 PFU MS2 aerosolized per meter traveled by the spray applicator. This is greater aerosolization than was observed during land application of biosolids. Because concentrations of coliphages and coliforms were similar in the liquid biosolids and the seeded water, it was concluded that some property of biosolids reduces aerosolization of microorganisms relative to groundwater. Additional experiments utilizing a novel air sampling protocol showed that the duration of bioaerosol exposure immediately (2 m) downwind of biosolids spray application is brief and the plume of bioaerosols generated is discrete. Additional air samples showed that aerosolization of coliphages and coliform bacteria after liquid biosolids have been applied to land does not occur at detectable levels.

* Corresponding author phone: (520)626-9284; fax: (520)621-6163; e-mail: benjamin.tanner@gmail.com.
† University of Arizona.
‡ Drexel University.

Introduction

The United States Environmental Protection Agency (USEPA) estimates that 6.9 million tons of Class B biosolids are generated in the United States each year, and that, by 2010, the amount generated per year will increase to 8.2 million tons (*1*). Of biosolids generated in the United States, approximately 60% are used as an organic amendment or land applied as a fertilizer (*2*). In some areas, Class B biosolids are applied to land as a liquid containing approximately 8% total solids. However, Class B biosolids typically contain enteric pathogens (*2*−*4*). As such, there has been speculation and public concern regarding the potential for aerosolization of pathogens during land application of biosolids.

Only one study has evaluated aerosolization of microorganisms resulting from land application of liquid biosolids. The study measured concentrations of coliphages, total coliform bacteria, fecal coliform bacteria, and fecal streptococci downwind of spray application of liquid biosolids using a large-volume electrostatic precipitator (*4*). These fecal indicator microorganisms were found intermittently and in low concentrations in air downwind of liquid biosolids application, but little information about the rate of aerosolization of microorganisms, the dimensions of the bioaerosol plume, or the duration of exposure to bioaerosols was obtained.

The current study was designed to determine the rate of aerosolization of microorganisms during land application of liquid biosolids, and to determine the duration of exposure to those bioaerosols. In addition, we sought to test the hypothesis that during application of liquid biosolids, microorganisms in the liquid phase comprise most of the bioaerosols generated by land application of biosolids, as opposed to those organisms adhering to solid particles. To test this hypothesis, air samples were taken downwind of land-applied groundwater, which had been seeded with coliphage MS2 and *E. coli* at concentrations similar to that found in liquid biosolids. Studies by Jakubowski et al. (*5*), Bitton et al. (*6*), and Chetochine et al. (*7*) on the transport of phage from biosolids through soil indicate that large numbers of phage are adsorbed or embedded within biosolids. By applying groundwater containing concentrations of MS2 and *E. coli* similar to concentrations found in biosolids, we were able to assess the role that solids play in aerosolization of microorganisms. Additionally, seeded groundwater provided greater aerosolization of microorganisms than liquid Class B biosolids under similar environmental and application parameters and served as a model system permitting characterization of the dimensions of the bioaerosol plume and the duration of exposure to bioaerosols generated during land application of liquid biosolids.

Materials and Methods

Air Samples. Air samples were collected using SKC Biosamplers (SKC, Inc., Eighty-Four, PA). The vacuum for each sampler was provided by factory calibrated Vac-U-Go vacuum pumps (SKC, Inc.), such that each air sampler impinged air at the rate of 12.5 L/min. Impingement buffer consisted of 23 mL of sterile water containing 0.1% peptone (Difco, Inc., Sparks, MD) and 0.1% antifoam B emulsion (Sigma Chemical

10.1021/es0404466 CCC: $30.25

Published on Web 02/11/2005

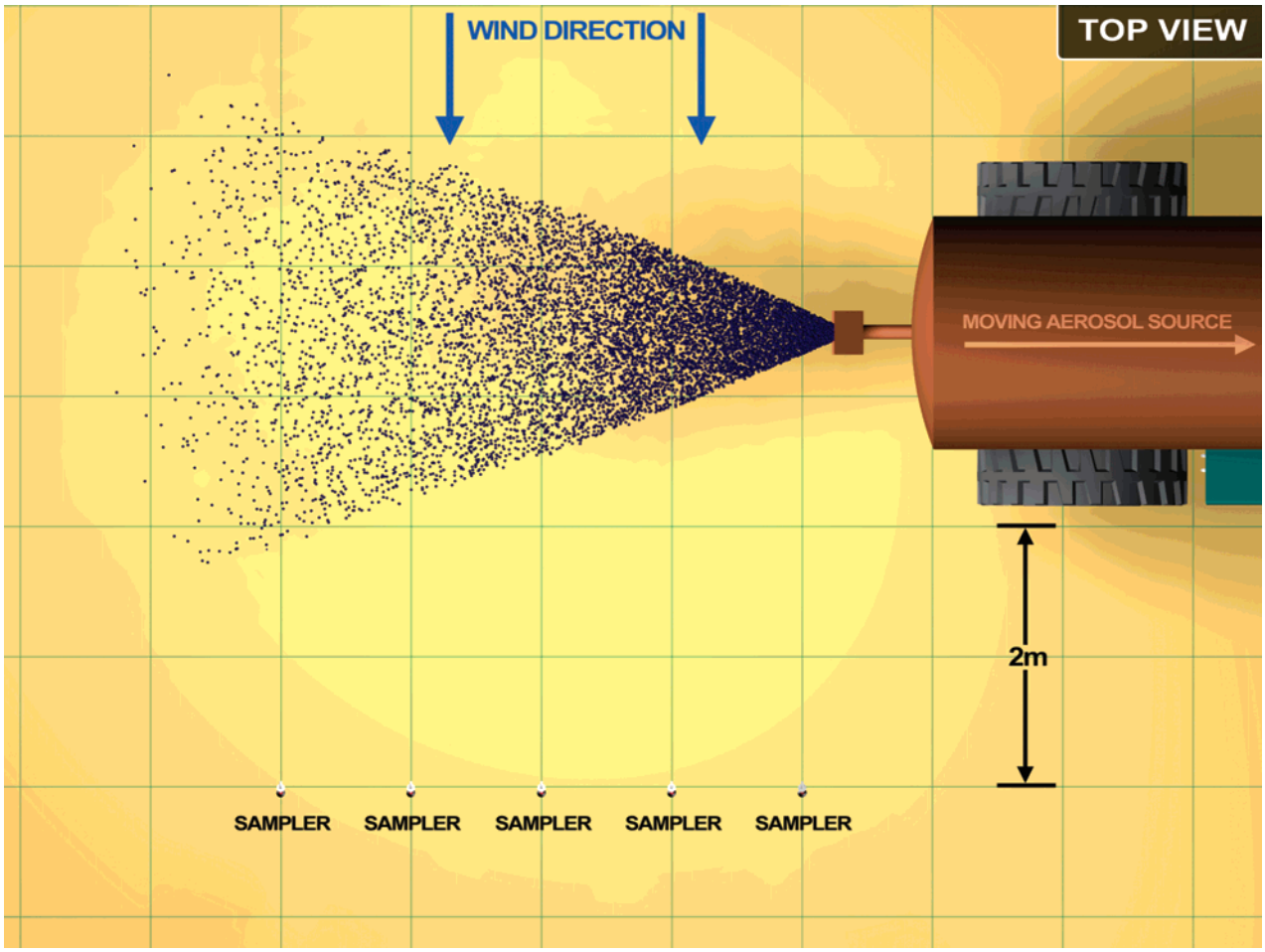

FIGURE 1. Air sampler placement.

Co., St. Louis, MO) (*8*). The final yield of impingement buffer varied, but was always more than 14 mL. Microbial concentrations in impingement buffer were inputted into an Excel spreadsheet that took into account the concentration of the sample by evaporation and calculated the concentration of microorganisms in air. Limits of detection were calculated as the pooled limit of detection of five air samplers, accounting for the average buffer volume after evaporation (18.2 mL, empirically determined from this study), the rate of air impingement (12.5 L/min), air sample duration (20 min), and amount of final buffer analyzed for a given assay (10 mL for total coliforms, 3 mL for coliphage, and 0.1 mL for HPC bacteria).

Air Sampler Arrangement and Air Sample Duration. Air samples were collected in a linear array consisting of five or six air samplers, positioned 2 m downwind of the edge of the spray of biosolids or seeded groundwater, arranged either horizontally (side by side) or vertically (one above the other). For air samples taken in horizontal arrangement, each air sampler was mounted on a tripod. Air samplers were aligned parallel to the direction of travel of the passing spray applicator and approximately perpendicular to the direction of the wind (Figure 1). Tripods were adjusted such that the air intake was facing the wind, positioned at a height of 1 m. Vertical samples were taken during application of seeded groundwater by affixing an air sampler at ground level, and air samplers at heights of 1, 2, 3, 4, and 5 meters, to a 5 m length of poly(vinyl chloride) (PVC) tubing (5 cm diameter). Air samplers were affixed to the PVC tubing using a 0.5 cm copper pipe which was passed through a hole drilled in the PVC and hose-clamped to the air sampler so that the air sampler rotated and remained vertical whether the PVC pole was horizontal (for sample loading and collection) or vertical (for collection of air samples). With the exception of the "time-based experiments" below, air sampling began 1 min before the passage of a 1.61×10^4 L capacity splash-plate spray applicator (Betterbuilt, Inc., Kalida, OH) and was collected for a 20 min period.

For this study, a typical land application event involved a spray-tanker land applying biosolids at a speed of approximately 2.2 m/s (5.0 miles/h), for a distance of 1000 m in a series of 10 "passes" of 100 m.

On one occasion, air samples were collected 1 day after biosolids had been applied to land to assess re-aerosolization of microorganisms using a horizontal array of air samplers used in the same fashion as during land application. These samples were collected under moderately windy conditions (2.4 m/s).

To establish the duration of exposure to aerosols as the spray applicator passed, three different sampling regimens were employed and are called, collectively, the "time-based experiments". Time-based experiments were conducted on three different days, downwind of land application of seeded groundwater. Each time-based experiment was conducted during a 2 h period and consisted of three distinct sampling regimens, performed as follows: For the first experiment, air sampling was started 60 s after the applicator had passed by the air samplers and continued for a total of 20 min. For the second experiment, air samplers were turned on 10 s before the spray applicator passed the line of samplers and continued for 1 min. For the third experiment, air samplers were turned on 10 s before the applicator reached the air samplers and air samples were collected for a 20 min period

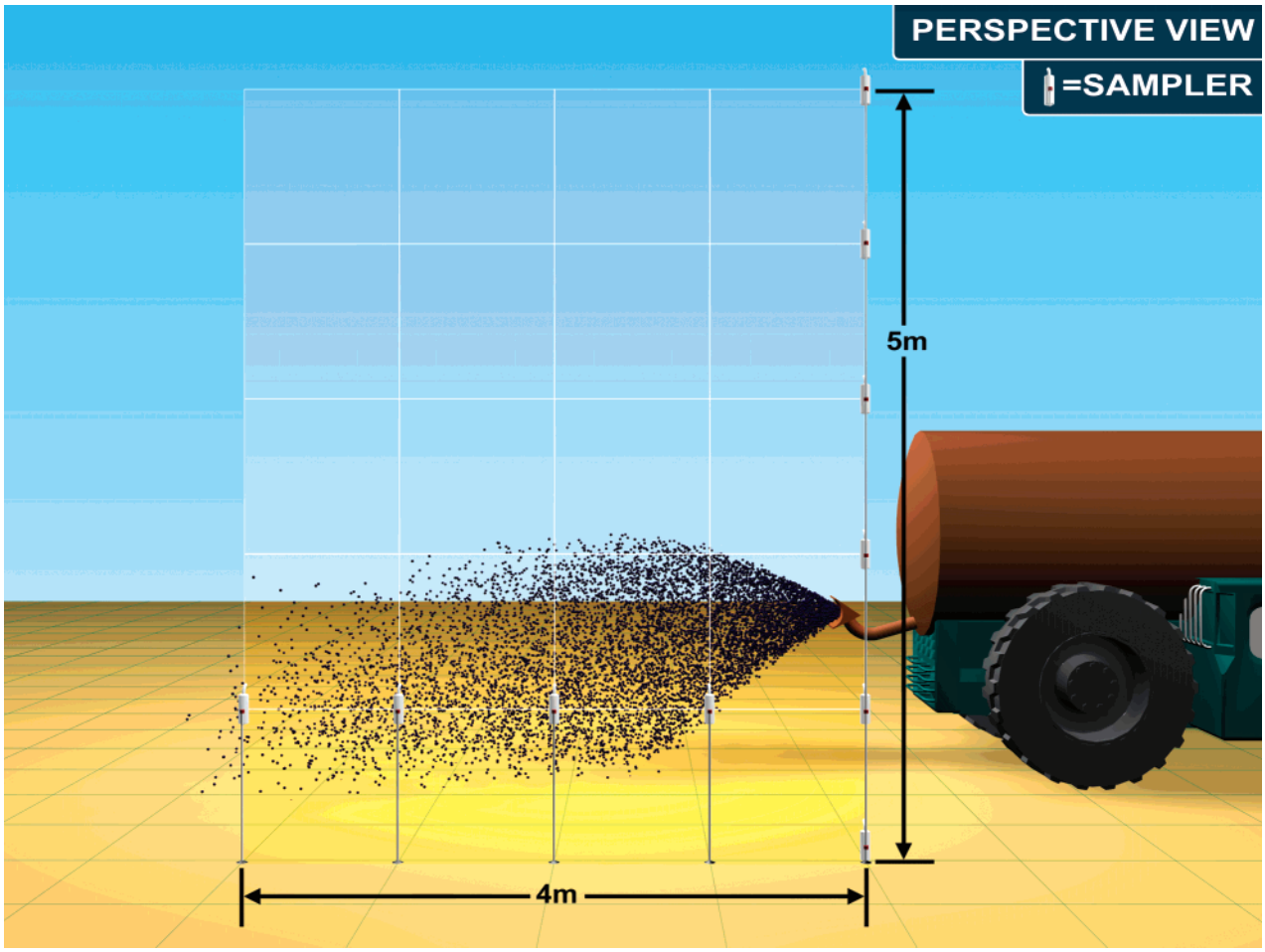

FIGURE 2. Aerosol flux.

TABLE 1. Environmental Conditions Encountered during Air Sampling

	average [range]		
sample date	windspeed (m/s)	temp (°C)	humidity (%)
2/19/03	0.7 [0.5−1.1]	22.4 [20.4−23.9]	19.0 [15.0−24.0]
3/05/03	1.0 [0.5−1.5]	20.6 [17.6−21.6]	19.0 [16.0−21.0]
2/17/03	1.1 [1.0−1.2]	26.6 [24.5−29.0]	24.3 [21.0−27.0]
3/05/03	1.6 [1.2−2.2]	13.1 [13.0−13.3]	52.0 [43.0−65.0]
3/12/03	1.0 [0.8−1.4]	30.1 [28.2−32.5]	6.3 [5.0−7.0]
6/11/03	1.7 [1.0−3.0]	33.7 [32.0−34.9]	4.3 [3.0−5.0]
6/18/03	1.7 [0.6−3.0]	38.3 [38.0−38.9]	8.3 [7.0−10.0]

after the applicator passed. All time-based experiments were conducted using air samplers in a horizontal array.

Environmental Data. Environmental data, including windspeed, temperature, and relative humidity, were collected using a Kestrel 3000 Pocket Weather Station, (Nielsen-Kellerman Co., Chester, PA) (Table 1).

Characteristics of Biosolids. All biosolids considered for this study were produced by mesophilic anaerobic digestion of municipal sewage sludge. Biosolids were obtained from the Roger Road Wastewater Treatment Plant (Tucson, AZ) and the Ina Road Wastewater Treatment Plant (Tucson, AZ). Biosolids were not stored prior to use.

Microbiological Preparation for Seeded Groundwater Studies. *E. coli* (ATCC 25922) was obtained from the American Type Culture Collection, Rockville, MD. It was maintained on Tryptic Soy Agar (TSA) (BD Diagnostics, Sparks, MD) and propagated from a colony in four aliquots of 100 mL of Tryptic Soy Broth (TSB) (BD Diagnostics) at 37 °C for 8 h. The resultant broth containing approximately 10^9 *E. coli*/mL then served as the inoculum for four sterile plastic 20 L buckets, each of which contained 16 L of sterile TSB at 37 °C. The inoculated buckets were placed in a walk in incubator and stirred at 37 °C for 24 h. The solution was then refrigerated for 12 h at 4 °C before addition to the spray applicator (Betterbuilt, Inc.).

MS2 coliphage (ATCC strain 15597-B1) was propagated as follows: A host culture of *E. coli* ATCC strain 15597 was prepared by overnight incubation in 100 mL of TSB on a shaker at 37 °C. Aliquots of 10^5 MS2 were added to test tubes containing 5 mL of overlay agar (30 g of TSB agar plus 10 g of Bacto agar, Difco, Detroit, MI) to which 1.0 mL of *E. coli* 15597 culture had been added. The mixture was poured onto TSA plates and incubated for 12 h. Following incubation, 10 mL of sterile Tris-buffered saline solution (Trizma base, Sigma Chemical Co., St. Louis, MO) was added by pipet to the Petri dishes and allowed to incubate at room temperature for 2 h. This buffer was then collected by pipet and centrifuged at 7000*g* for 10 min to remove cell debris and filter sterilized using a 0.22 micrometer pore size filter (Millipore, Inc., Billerica, MA).

Operation and Seeding of the Spray Applicator. The spray applicator consisted of a large tank towed behind a tractor. It used pressurized air to force biosolids or seeded groundwater through a 7.5 cm diameter pipe against a metal plate at a height of approximately 1.5 m, causing the liquid to disperse as a "fan" shape, reaching a maximum height of 2 m (Figure 2). For experiments using seeded groundwater, the spray applicator was rinsed twice by filling and discharging groundwater, and then was filled with approximately 1.0×10^4 L groundwater. Approximately 5.0×10^{12} *E. coli* and

approximately 10^{14} MS2 coliphage were added to the groundwater contained in the spray applicator. This resulted in concentrations of coliphage in the applicator ranging from 5.5×10^5 to 4.0×10^7 pfu/mL and concentrations of *E. coli* ranging from 9.6×10^4 to 2.3×10^7 cfu/mL. The tractor driver thoroughly mixed the inoculum by towing the applicator over irregular terrain for approximately 5 min. Seeded groundwater was then applied to land in the same manner as liquid biosolids, and air samples were taken downwind. Aliquots of the seeded groundwater were collected at the time of application for laboratory analysis of *E. coli* and coliphage concentrations in the spray applicator.

Microbiological Assay of Air Samples. Total coliform bacteria and *E. coli* were enumerated by the membrane filter method on m-Endo agar (BD Diagnostics, Sparks, MD) at 37 °C for 24 h in accordance with Standard Method 9222B (*9*). One 10 mL aliquot of impingement buffer from each air sampler was assayed for the presence of total coliforms or *E. coli* in each experiment.

MS2 coliphage, and all other coliphage capable of infecting *E. coli* 15597-B1, were assayed by the agar overlay method (*10*). A host culture of *E. coli* ATCC strain 15597 was prepared by overnight incubation in 100 mL of TSB on a shaker at 37 °C. A 1 mL aliquot of air sample impingement buffer from each air sampler was added to a test tube containing 5 mL of overlay agar, to which 0.5 mL of *E. coli* 15597 culture had been added. The mixture was poured onto TSA plates, incubated overnight, and enumerated. This assay was performed in triplicate.

Microbiological Assay of Biosolids. Coliphages and coliform bacteria in biosolids were assayed as follows: 10 mL of liquid biosolids was placed in 95 mL of sterile Tris-buffered saline solution (Sigma) and shaken for 30 min at room temperature. The resulting mixture was then serially diluted in sterile Tris-buffered saline (Sigma) and assayed directly for coliphages using the agar overlay method (*10*). Total coliform bacteria, including *E. coli*, were enumerated by the spread plate technique on m-Endo agar (BD Diagnostics). Total percent solids was determined by drying the biosolids in an oven overnight at 105 °C, in accordance with Standard Method 2450-B (*11*).

Calculation of the Rate of Emission of Microorganisms from the Spray Applicator. The virtual plane is created by the mathematical combination of values from horizontal and vertical air samples. Calculation of flux through this plane, which is 4 m wide by 5 m high (Figure 2), was performed using the following equation:

$$F = (C \times V)_{\text{avg}} \times W \times H$$

Calculation of the rate of emission of aerosolized microorganisms was performed using the following equation:

$$E = (C \times V)_{\text{avg}} \times H$$

where

F is the flux, the average number of microorganisms that pass through the 20 m^2 virtual plane of samplers per second during the 1200 s (20 min) air sample collection period;

E is the emission source strength, the average number of indicator microorganisms aerosolized by the spray applicator per meter traveled by the applicator per second during the 1200 s air sample collection period;

C is the average downwind concentration (microorganisms/m^3) determined by air samples;

W is the width of the "plane" of air samplers (m);

H is the height of the "plane" of air samplers (m); and

V is the approximately perpendicular velocity of wind passing through the "plane" (m/s). The average V for this study was 1.3 m/s.

Results and Discussion

Environmental Conditions. Table 1 shows the environmental conditions encountered during the study. In general, conditions were sunny, hot, and dry and windspeed was variable, which is normal in the Desert Southwest. Notably, on one day involving land application of seeded groundwater, temperatures were recorded above 38 °C, which is a higher temperature than is experienced in most parts of the United States, even during the summer. These environmental conditions may have influenced the emission rate of microorganisms calculated in this study by causing inactivation of some portion of aerosolized microorganisms during their brief airborne transport to the samplers. Research has demonstrated that gram negative bacteria, such as *E. coli*, quickly lose culturability and viability after aerosolization at low (20−25%) relative humidities (*12*). The relative humidity was, on average, 16% for this study.

Emission Rate of Aerosolized Microorganisms during Land Application of Biosolids. To estimate the maximum rate of aerosolization that could occur without detection by our samplers, the limits of detection for our methodology were calculated to be 1.5 CFU total coliforms and 5.1 PFU coliphage per m^3 air. No coliphages nor coliform bacteria were detected in bioaerosols during land application of biosolids in this study, although samplers were positioned directly downwind of the aerosol source and positioned at a height of 1 m (Figure 1). Thus, concentrations of coliphage and coliforms in air immediately downwind of spray application of liquid biosolids were below the limit of detection, although the liquid impingers chosen for the study have been shown to be efficient for the collection of aerosolized microorganisms (*13*, *14*). It should be noted that this study only considered a land application site in the Southwest and may not be representative of other land application sites. However, the results of this study were similar to previous research, which was unable to detect coliphages using an electrostatic air sampler and reported median concentrations of total coliform bacteria ranging from nondetectable to 82 cfu/m^3 (*4*).

By substituting the limits of detection for the concentrations of coliform bacteria and coliphage in air, the rate of aerosolization of coliform bacteria during land application of liquid biosolids was shown to be less than 10 cfu/m/s and the rate of aerosolization of coliphage was shown to be less than 33 pfu/m/s (where meters are those traveled by the tractor and seconds are those which elapse during the sample collection period). At this rate of aerosolization, a tractor spray-applying liquid Class B biosolids for 1000 m (1200 s air sample duration) would aerosolize, at most, approximately 1.2×10^7 coliforms and 4.0×10^7 coliphage. Importantly, we were unable to detect coliform bacteria or coliphages in air at the land application sites the day after biosolids had been applied, even under moderately windy conditions (2.4 m/s), using air samplers set up in the same fashion as during land application. This suggests that re-aerosolization of microorganisms does not occur to a great extent after land application of biosolids.

Emission Rate of Aerosolized Microorganisms during Land Application of Seeded Water. Concentrations of *E. coli* ranged from 9.6×10^4 cfu/mL to 2.3×10^7 cfu/mL and concentrations of MS2 ranged from 5.5×10^5 pfu/mL to 4.0×10^7 pfu/mL in the groundwater in the spray applicator during experiments where seeded groundwater was applied in the same manner as biosolids (Table 2). This was an appropriate reproduction of the concentrations of coliphage and coliform bacteria, which have recently been shown to

TABLE 2. Average Concentrations of Coliforms and Coliphage in Biosolids, Seeded Groundwater, and Air Downwind of Land Application[a]

				concentration in air		concentration in liquid	
date	air samples taken	applied to land	air sampler array	coliforms (cfu m^{-3})	coliphage (pfu m^{-3})	coliforms (cfu mL^{-1})	coliphage (pfu mL^{-1})
2/19/03	15	liquid biosolids	horizontal	1.5	5.1	2.0×10^{5} [b]	1.4×10^{4} [b]
3/05/03	15	liquid biosolids	horizontal	1.5	5.1	2.0×10^{5} [b]	1.4×10^{4} [b]
2/17/03	15	seeded water	horizontal	14	30	1.4×10^{7}	5.3×10^{6}
3/05/03	15	seeded water	horizontal	4.0	11	4.1×10^{6}	9.0×10^{5}
3/12/03	15	seeded water	horizontal	3.0	157	2.3×10^{7}	2.8×10^{7}
6/11/03	18	seeded water	vertical	56	5.1	1.1×10^{5}	5.5×10^{5}
6/18/03	16	seeded water	vertical	1.5	19	4.0×10^{5}	4.0×10^{7}

[a] When microorganisms were not detectable in an air sample, the limit of detection was used to represent the concentration of microorganisms in air for that sample. [b] Average value from a composite of three biosolids samples from different batches of biosolids collected on 02/19/03 and 03/05/03, and assayed on 03/05/03.

TABLE 3. Flux and Emission Rate of Bioaerosols during Land Application

source	flux, *F* (CFU or PFU/s)	emission rate, *E* (CFU or PFU/m·s)
	Coliforms/*E. coli*	
biosolids[a]	3.9×10^{1}	1.0×10^{1}
seeded groundwater[b]	8.09×10^{3}	2.0×10^{3}
Coliphage/MS2		
biosolids[a]	1.3×10^{2}	3.3×10^{1}
seeded groundwater[b]	1.5×10^{4}	3.9×10^{3}

[a] Calculations were based on the limit of detection of the air samplers from samples taken at 1 m only. Parameters were defined as follows: $C = 5.1$ (coliforms), $C = 1.5$ (coliphage), $H = 5$, $V = 1.3$ m/s, and $W = 4$ m. [b] Calculations were based on the average microbial concentrations observed in horizontally aligned samples taken on 02/17/03, 03/05/03, and 03/12/03, and vertically aligned samples taken on 06/11/03 and 06/18/03 from Table 2. Parameters were defined as follows: $C = 311$ (coliforms), $C = 594$ (coliphage), $H = 5$, $V = 1.3$ m/s, and $W = 4$ m.

be present in biosolids (*7*). The recent research, involving multiple, sequential assays of coliphages in biosolids, demonstrated that the true concentration of coliphage in liquid biosolids is approximately 2 orders of magnitude greater than that typically measured by a single coliphage assay from liquid biosolids, as was performed in this study.

Land application of groundwater seeded with *E. coli* and MS2 produced significantly more aerosols than land application of liquid biosolids, as measured by a one tailed *t*-test using Microsoft Excel ($\alpha = 0.05$, $p < 0.001$). The concentrations of microorganisms observed per cubic meter of air downwind of spray application of seeded groundwater is shown for samples taken in horizontal and vertical arrangement (Table 2). Flux and emission rate were also calculated (Table 3). The greater aerosolization that was observed during land application of seeded groundwater, relative to that which was observed during biosolids application, indicates that some physical property of the biosolids is probably responsible for the reduction in aerosolization of bacteria and viruses. It has been shown that viruses are predominantly adsorbed to particulate matter in biosolids (*6*, *7*). Accordingly, it is logical that aerosolization of viruses including coliphages would be increased by the elimination of heavy, virus adsorbing particles which are subject to rapid gravitational settling. It is likely that the particulate matter in biosolids would also provide adsorption sites for bacteria, thereby limiting their aerosolization in a fashion similar to viruses.

Notably, more coliphages than coliforms were detected in air downwind of land application of seeded groundwater, even when the inoculum contained similar concentrations of both organisms. This may be due to rapid loss of culturability or viability by bacteria in air.

Characterization of the Bioaerosol Plume Using Seeded Groundwater. Information about the plume of bioaerosols created during land application of liquid biosolids is important. The height of the plume is related to the potential for transport of microorganisms over long distances. The width of the plume, which can be thought of as the distance from the leading edge of the plume to the lagging edge of the plume with respect to an individual downwind, is related to the duration of exposure. To provide information about these previously unknown dimensions, the aerosol plume generated during land application of biosolids was characterized using land application of seeded water as a model. The spray generated during land application of seeded water was similar to the spray generated during land application of liquid Class B biosolids and served as a conservative model for estimating the fate of indicator microorganisms due to liquid Class B biosolids application (Figure 3).

Vertically arranged samples were taken in six sets on two different days during land application of groundwater seeded with coliphage MS2 and *E. coli*. In total, 34 air samples were taken in this manner, with air samplers spanning from 0 to 5 m in height for each of the six sets of samples. Neither coliforms nor coliphages were detected above 3 m in height, and both types of microorganisms were most frequently detected at heights of 2 m or below. This suggests that the maximum height of aerosolized microorganisms is no more than 3 m at the aerosol source under the environmental conditions observed during this study. Interestingly, vertical air samples revealed that the majority of aerosols containing *E. coli* are found between ground level and 1 m, whereas coliphage were readily detectable at heights up to 3 m above the ground. This suggests that coliform bacteria were either losing culturability and/or being inactivated immediately after aerosolization, or were settling more quickly than coliphage because they were contained within larger droplets of water. Consequently, one may expect the transport of aerosolized viruses to occur to a greater extent than the transport of aerosolized gram negative bacteria.

FIGURE 3. Biosolids spray (left) and seeded groundwater spray (right).

TABLE 4. Total Capture of *E. coli* in Air Samplers during Time-Based Experiments Using Seeded Groundwater[a]

date	20 min air sample, started 1 min after pass of spray applicator (CFU)	1 min sample taken during pass of applicator (CFU)	normal, 20 min sample started just before pass of applicator (CFU)
03/16/03	0	0	4
06/25/03	0	2	0
09/03/03	0	0	0
total	0	2	4

[a] Each value represents the sum of coliforms captured and cultured from five separate air samplers, running in unison.

TABLE 5. Total Capture of MS2 Coliphage in Air Samplers during Time-Based Experiments Using Seeded Groundwater[a]

date	20 min air sample, started 1 min after pass of spray applicator (PFU)	1 min sample taken during pass of applicator (PFU)	normal, 20 min sample started just before pass of applicator (PFU)
03/16/03	0	3	14
06/25/03	0	21	8
09/03/03	0	46	21
total	0	70	43

[a] Each value represents the sum of coliphage captured and cultured from five separate air samplers, running in unison.

Previously published research, designed to assess the risk of infection due to aerosolized pathogens from biosolids, considered biosolids to be a continuous source of exposure for 8−24 h (*8*). To investigate this assumption, we conducted time-based experiments. These experiments provided an estimation of the width of the plume and potential duration of exposure to bioaerosols produced during land application of liquid biosolids. Results are presented as the sum of organisms detected in air sampler impingement buffer, rather than as concentrations per cubic meter of air, to facilitate comparison of these data (Tables 4 and 5). Clearly, fewer coliforms than coliphage were detected, even though concentrations in the seeded groundwater were similar on average. Time-based experiments indicate that a point 2 m downwind of the spray applicator is exposed to bioaerosols for less than 1 min per pass of the applicator. Thus, the spray applicator is a moving, continuous source of aerosols, but exposure of a stationary individual to aerosols would only occur during a discrete pulse of short duration, just after the tractor passes. A typical application of biosolids, in our estimation, requires approximately 10 "passes". Therefore, the maximum duration of exposure to a stationary individual would be less than 10 min. Thus, the duration of exposure to aerosolized microorganisms is far less than 24 h, which has been used in previous studies as an estimate of the duration of exposure to bioaerosols from land application (*8*).

Implications for Aerosolization of Pathogens during Land Application of Biosolids. The primary concern with respect to aerosols generated during land application of liquid biosolids is the potential for aerosolization of pathogenic viruses and bacteria contained within the biosolids. Detection of enteric pathogens in air downwind of biosolids is challenging because expensive and specific assays must be performed to detect the array of pathogens potentially present in aerosols. To overcome this problem, and to maximize our limit of detection of aerosolized bacteria and viruses, we analyzed a large portion (10 mL) of our collection buffer for the presence of total coliform bacteria and another portion (3 mL) for coliphage. We then used what is known about the microbial composition of biosolids to estimate aerosolization of pathogens during land application.

Current literature and research recently performed in our laboratory suggests that there is approximately one human pathogenic bacterium per 1000 coliform bacteria in biosolids (*2*−*4*). Because of similarities in size and structure, it follows that approximately 1000-fold less pathogenic bacteria are aerosolized during land application of biosolids than coliform bacteria. Because we estimate that 1.2×10^7 coliforms are aerosolized during a typical land application of biosolids wherein the spray applicator travels 1000 m, we can reasonably assume that approximately 1.2×10^4 pathogenic bacteria would be aerosolized during the same application. This reasoning can also be used to estimate aerosolization of enteric viruses during land application of liquid biosolids. According to current literature and studies performed in our laboratory, there are approximately 1000 times more coliphage than human enteric viruses in Class B biosolids (*2*−*4*). Therefore, total aerosolization of human enteric viruses during a typical liquid biosolids application is likely to be 1000-fold less than our estimation of the total aerosolization of coliphages of 4.0×10^7, suggesting that approximately 4.0×10^4 enteric viruses would be aerosolized during a typical application of biosolids.

Based on these rates of aerosolization of microorganisms during land application of liquid biosolids, the concentration of aerosolized pathogens per cubic meter of air is low. For example, for a spray applicator applying liquid biosolids for 1000 m with a windspeed of 2.0 m/s, pathogens would be aerosolized within 1.2×10^7 m^3 air. This volume is calculated as a large cube, with height corresponding to the maximum height of the vertical sampling apparatus used in the study, width corresponding to the 1000 m traveled by the spray

applicator, and length corresponding to the distance that the air travels during our 20 min sample, which is calculated by multiplying the average windspeed by the duration of our air sample, as shown in the following calculation: 2.0 $m/s_{windspeed} \times 1200\ s_{sampleduration} \times 1000\ m_{traveled} \times 5\ m_{height} = 1.2 \times 10^7\ m^3$. By dividing the total number of pathogens suspected to be aerosolized during land application of biosolids by the total potentially contaminated volume of air, the concentration of viral pathogens per cubic meter of air was calculated as follows: 4.0×10^4 viral pathogens $\times$ $1.2 \times 10^7\ m^3\ air^{-1} = 3.3 \times 10^{-3}$ viral pathogens/m^3. Thus, airborne viral pathogens resulting from land application of biosolids should be present at concentrations of no more than approximately 3.0×10^{-3} pathogenic viruses per cubic meter of air, downwind of the aerosol source. Similarly, the concentration of bacterial pathogens in air resulting from land application of biosolids was calculated as follows: 1.2×10^4 bacterial pathogens $\times$ $1.2 \times 10^7\ m^3\ air^{-1} = 1.0 \times 10^{-3}$ bacterial pathogens/m^3. Thus, airborne bacterial pathogens resulting from land application of biosolids are present at concentrations of no more than approximately 1.0×10^{-3} pathogenic bacteria per cubic meter of air, immediately (2 m) downwind of the aerosol source. The human breathing rate is estimated to be approximately 0.675 m^3/h for adults (*15*). Therefore, the probability of inhaling an aerosolized enteric pathogen immediately downwind of land-applied Class B biosolids is likely to be very low for short-term exposure scenarios. In addition, this calculation neglected dissipation of pathogens in air above 5 m in height and neglected to account for inactivation of the pathogens due to environmental factors. Also, inhalation of many enteric pathogens has not been shown to result in infection.

In conclusion, aerosolized microorganisms were not detectable during land application of liquid Class B biosolids near Tucson, Arizona. Land application of seeded water was a useful experimental tool and demonstrated that the aerosol plume generated during land application is detectable from a stationary point for no more than 1 min per "pass" of the applicator. Thus, exposure to bioaerosols containing coliform bacteria and coliphages resulting from land application of liquid class B biosolids is discrete and occurs at low concentrations. This study also suggests that exposure to aerosolized pathogenic microorganisms is lower than previously estimated. Our results are noteworthy because we relied on direct measurements taken near the source, rather than measurements taken at great distances and manipulated using dispersion models, to estimate the rate of aerosolization of microorganisms during biosolids application (*16*).

Acknowledgments

This work was supported by The University of Arizona, National Science Foundation Water Quality Center, in Tucson, Arizona. We thank Mike Martelle, from the Learning Technologies Center at the University of Arizona, for rendering Figures 1 and 2.

Literature Cited

(1) United States Environmental Protection Agency (USEPA). Municipal and Industrial Solid Waste Division, Office of Solid Waste. *Biosolids Generation in the United States*, EPA530-R-99-009; 1999.

(2) National Research Council (NRC). Committee on Toxicants and Pathogens Applied to Land. *Biosolids Applied to Land: Advancing Standards and Practices*, ISBN 0-309-08486-5.

(3) Straub, T. M.; Pepper, I. L.; Gerba, C. P. Hazards from pathogenic microorganisms in land-disposed sewage sludge. *Rev. Environ. Contam. Toxicol.* **1993**, *132*, 55−91.

(4) Sorber, C. A.; Moore, B. E.; Johnson, D. E.; Harding, H. J.; Thomas, R. E. Microbiological aerosols from the application of liquid sludge to land. *J.-Water Pollut. Control Fed.* **1984**, *56*, 830−836.

(5) Jakubowski, W.; Galke, W.; Love, G. USEPA. Virus transport through soil. EPA-600/1-79-015; 1979.

(6) Bitton, G.; Pancorbo, O. C.; Farrah, S. R. Virus transport and survival after land application of sewage sludge. *Appl. Environ. Microbiol.* **1984**, *47*, 905−909.

(7) Chetochine, A. S. Retention of phage by class B biosolids and potential transport through soil. Master's Thesis, University of Arizona Department of Soil, Water and Environ. Science, 2003.

(8) Dowd, S. E.; Gerba, C. P.; Pepper, I. L.; Pillai, S. D. Bioaerosol transport modeling and risk assessment in relation to biosolid placement. *J. Environ. Qual.* **2000**, *29*, 343−348.

(9) Franson, M. A. H. In *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; Eaton, A. D., Clesceri, L. S., Greensberg, A. E., Eds.; Method 9222B; 1995.

(10) Adams, M. H. *Bacteriophages*; Interscience Publishers: New York, 1959.

(11) Franson, M. A. H. Total Solids Dried at 103−105 °C. In *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; Eaton, A. D., Clesceri, L. S., Greensberg, A. E., Eds.; Method 2540C; 1995.

(12) Heidelberg, J. F.; Shahamat, M.; Levin, M.; Rahman, I.; Stelma, G.; Grim, C.; Colwell, R. R. Effect of aerosolization on culturability and viability of gram-negative bacteria. *Appl. Environ. Microbiol.* **1997**, *63*, 3585−3588.

(13) Lin, X.; Willeke, K.; Ulevicius, V.; Grinshpun, S. A. *Am. Ind. Hyg. Assoc. J.* **1997**, *58*, 480−488.

(14) Willeke, K.; Lin, X.; Grinshpun, S. A.; Reponen, T. Laboratory and field evaluation of a new bioaerosol sampling concept. *J. Aerosol Sci.* **1998**, *29*, S499−S500.

(15) Allan, M.; Richardson, G. M. Probability density functions describing 24-hour inhalation rates for use in human health risk assessments. *Hum. Ecol. Risk. Assess.* **1998**, *4*, 379−408.

(16) Sawyer, B.; Elenbogen, G.; Rao, K. C.; O'Brien, P.; Zenz, D. R.; Lue-Hing, C. Changes in bacterial aerosols with height above aeration tanks. *Appl. Environ. Microbiol.* **1993**, *59*, 3183−3186.

Received for review June 18, 2004. Revised manuscript received November 18, 2004. Accepted December 16, 2004.

ES0404466