TECHNICAL NOTE:

EFFECT OF SPRINKLER PRESSURE AND SPRAY PLATE ON CULTURABLE MICROORGANISM CONCENTRATIONS DURING SIMULATED IRRIGATION OF DAIRY WASTEWATER

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ABSTRACT. *In this study, we conducted simulated spray irrigation events of dairy wastewater to assess the impact of pressure and sprinkler type on post-sprinkler culturable microorganism concentrations. Dairy wastewater was sampled before and after it was pumped through sprinklers typically used on center-pivot irrigation systems. Three different sprinklers types were used at three different operating pressures to give a range of water drop sizes. The microorganisms quantified in this study were total coliforms,* Escherichia coli*,* Clostridium perfringens*, heterotrophic bacteria, and coliphages. In most cases, the pre- and post-sprinkler concentrations were determined to be statistically similar, suggesting that culturable viability was not affected when wastewater flowed through these sprinklers. When an impact was found to occur, there was usually an increase in the post-sprinkler microorganism concentration. While this increase can be attributed to the disruption of microbial aggregates during the spraying process, there was no apparent relationship with pressure setting or spray plate. Understanding impacts at the sprinkler level should be considered an integral part of the dispersion modeling process, as it may influence the number of viable microorganisms that become aerosolized during pressurized irrigation events.*

Keywords. *Culturable, Dairy, Irrigation, Microorganism, Sprinkler, Wastewater.*

Concentrated animal feeding operations (CAFOs) generate large quantities of feces and urine that are typically stored at the facility until they are either treated or beneficially used in some manner (Rice et al., 2006). The animal wastes are frequently applied to land as a cost-effective means for disposal, although additional benefits are an increase in the soil nutrient status (Sims et al., 2005). While manure solids are often applied using a box-type spreader, liquid wastes are typically broadcast or directly injected using a tank-type applicator or spread using sprinkler irrigation (Pfost and Fulhage, 2001). Use of these land application techniques enhances the aerosolization of zoonotic pathogens and microbial byproducts in the untreated manures, thus increasing the risk of exposure to farm workers and individuals in the downwind environment (Dungan, 2010).

Although there is a move to directly inject liquid wastes into soil to mitigate ammonia emissions (Morken and Sakshaug, 1998; Hansen et al., 2003; Lovanh et al., 2010), it is also common practice to pump liquid waste from storage tanks and ponds to pressurized irrigation systems (Sharpe and Harper, 1997). Investigations into the transport of aerosolized microorganisms during the spray application of animal wastes have shown a decrease in the airborne concentration with distance from the irrigation source, suggesting a low respiratory hazard for nearby individuals (Boutin et al., 1988; Hutchison et al., 2008). Murayama et al. (2010) used a PCR-based assay to identify culturable airborne microorganisms during the aerial spraying of a swine slurry and found that none of the organisms were described previously as having an inhalation route of infection. Decreases in the airborne microorganism concentration with downwind distance were also observed during spray irrigation events of municipal wastewaters (Katzenelson and Teltch, 1976; Bausum et al., 1982; Camann et al., 1988).

To assess the transport and diffusion of airborne microorganisms during wastewater irrigation events, models based on Pasquill's (1961) particle dispersion model have been utilized (Parker et al., 1977; Teltsch et al., 1980). Since the Pasquill model was based on inert particles, a biological decay term (λ) was added to account for the death of aerosolized microorganisms (Lighthart and Frish, 1976). In addition to λ, a dispersion model later used by the U.S. EPA (1982) included a microorganism impact factor (I), which was added to represent the initial shock associated with the aerosolization process (e.g., rapid evaporation of water, turbulent forces). To date, however, researchers have not defined the suspected individual components of I. The objective of this study was to assess post-sprinkler impacts on culturable microorganisms by pumping dairy wastewater through center-pivot sprinklers with a rotating or fixed (flat or grooved) spray plate under various pressures. The premise for this work was based on the hypothesis that increases in sprinkler pressure would cause reductions in microorganism survivability. This is the first assessment of I since the U.S. EPA (1982) work, with a specific focus on physical stresses prior to aerosolization of microorganisms.

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Materials and Methods

Wastewater Storage Pond

Wastewater from a storage pond at an open-freestall dairy with 10,000 Holstein cows was used for this study. Manure in the freestall barns was flushed daily from the alleys and then sent to a solids separator. Some of the liquid waste was utilized in an anaerobic digester system for biogas production. Undigested liquid waste, as well as digester effluent, was then stored in a series of ponds. The dairy commonly pumps the wastewater through center pivots to irrigate nearby fields.

Spray Chamber and Sprinkler

A spray chamber was used to contain the wastewater application from the sprinkler. The chamber was constructed from a 132 L opaque plastic drum (47 cm dia. × 76 cm high) by cutting a window (28 cm wide × 33 cm high) into the side of the drum. Galvanized steel piping (1.9 cm dia.) was installed through the top of the drum to allow a sprinkler to be mounted inside. The drum was mounted on a stand (55 cm high), and the piping was connected to a 0.56 kW centrifugal pump. After the centrifugal pump, a valve was installed so samples could be collected prior to the sprinkler. Pressure regulators were used to maintain water pressures of 138, 207, or 276 kPa at the sprinkler. A pressure gauge was installed before the pressure regulator to ensure that the water pressure was adequate for the test. Sprinklers (3000 Series, Nelson Irrigation Corp., Walla Walla, Wash.) were used with either a brown rotating plate (Part No. 10681), fixed flat smooth plate (Part No. 9591), or fixed concave grooved plate (Part No. 10721). A Nelson 3TN No. 21 nozzle (4.2 mm orifice dia.) was used, which produces flow rates of 12.7, 15.6, and 18 L min^{-1} at pressures of 138, 207, and 276 kPa, respectively.

Experimental Design

The spray chamber was placed near the edge of the wastewater pond, so the wastewater was sprayed back into the pond. A gas-powered generator was used to supply electricity to the centrifugal pump. Using a flotation device, the inlet end of the hose was suspended on the surface of the pond to avoid the intake of settled solids into the pump and clogging of the nozzle. Wastewater was pumped through the system for 5 min before collecting samples. A series of 10 pre- and post-sprinkler samples were then collected with approximately 2 min intervals between each collection. The samples were collected in sterile 500 mL plastic bottles and immediately placed into a cooler with ice packs. Enumeration of culturable microorganisms was conducted on the same day, usually within 8 h of sample collection.

Wastewater Analyses

The pH and temperature of the wastewater was determined *in situ* using a portable meter (model 260A, Thermo Scientific Orion, Beverly, Mass.). Total solids, dissolved solids, and total suspended solids were determined according to Standard Methods 2540b, 2540c, and 2540d, respectively (Eaton et al., 2005).

Enumeration of Microorganisms

The concentrations of total coliform, *Escherichia coli*, *Clostridium perfringens*, heterotrophic bacteria, and coliphages in the pre- and post-sprinkler samples were determined via cultivation. Prior to cultivation, 10-fold serial dilutions of the samples were prepared in phosphate-buffered saline. Total coliform and *E. coli* were assayed by filtering 100 mL aliquots through membrane filters (47 mm, 0.45 μm pore size) and incubating them at 35°C for 1 d on MI media (U.S. EPA, 2002). Vegetative cells and spores of *C. perfringens* were assayed using membrane filtration onto mCP media (Neogen, Lansing, Mich.) with mCP selective Supplement I (Sigma-Aldrich, St. Louis, Mo.), as described by Armon and Payment (1988). For the enumeration of clostridia spores, dilutions were heat-shocked at 80°C for 5 min prior to membrane filtration. The mCP plates were anaerobically incubated at 44.5°C for 1 d, with exposure to ammonia hydroxide vapors afterwards to quantify presumptive *C. perfringens* colonies. Heterotrophic bacteria were cultivated by spreading 0.1 mL aliquots on tryptic soy agar (TSA) media and incubating at 25°C for 5 d. Coliphages were assayed using the pour plate method with overnight incubation at 35°C (Eaton et al., 2005). Positive controls consisted of *E. coli* (ATCC 13706), *C. perfringens* (ATCC 13124), and coliphages (ATCC 13706-B1). All plates were manually counted, with bacterial and coliphage counts reported as colony forming units (CFU) and plaque forming units (PFU) per mL, respectively.

Statistical Analysis

Two-sample paired *t*-tests for the means were performed using SAS (2008). Statements of statistical significance were based on $p < 0.05$ unless otherwise stated.

Results and Discussion

During the sprinkler studies, the ambient air temperature ranged from 9.2°C to 25°C with a mean of 18.5°C. The relative humidity ranged from 19.3% to 84.1% with a mean of 42.8%. While the ambient weather data are not presented, select properties of the wastewater are presented in table 1. The mean pH and electrical conductivity were 7.7 and 12.3 mS m^{-1}, respectively. The wastewater temperature ranged from 13.7°C to 21.1°C with a mean of 18.2°C. The mean total solids, suspended solids, and total dissolved solids contents were 14,589, 7,055, and 8,000 mg L^{-1}, respectively.

The pre- and post-sprinkler microorganism concentrations under pressure settings of 138 and 207 kPa with rotating, flat, or grooved spray plates are presented in table 2. Due

Table 1. Properties of the dairy wastewater during the sprinkler study.

Sprinkler Pressure (kPa)	pH	EC (mS m^{-1})	Temp. (°C)	Total Solids	Suspended Solids (mg L^{-1})	Total Dissolved Solids
Rotating plate						
138	7.7	12.4	16.5	15,640	7,906	7,716
207	7.6	11.6	20.1	12,330	4,938	7,478
276	7.7	12.7	21.1	13,810	8,894	9,654
Fixed flat plate						
138	7.6	12.1	19.2	13,185	5,282	7,336
207	7.8	12.2	18.2	16,300	9,906	7,376
276	7.8	12.8	19.3	15,810	5,764	8,530
Fixed grooved plate						
138	7.8	12.6	13.7	17,485	10,672	6,934
207	7.9	12.3	18.5	14,260	5,428	9,280
276	7.7	11.9	17.4	12,480	4,702	7,692

Table 2. Mean culturable microorganism concentrations (CFU or PFU mL^{-1}) in pre- and post-sprinkler samples at pressure settings of 138 and 207 kPa.[a]

	138 kPa		207 kPa	
Microorganism	Pre-Sprinkler	Post-Sprinkler	Pre-Sprinkler	Post-Sprinkler
Rotating plate				
E. coli	433 (20)	408 (21)	1016 (28)	1154 (31)**
Total coliforms	3020 (271)	3220 (259)	3650 (212)	3360 (310)
C. perfringens (spore)	1185 (126)	1325 (53)	170 (26)	335 (51)**
C. perfringens (vegetative)	1990 (117)	1805 (106)	645 (75)	580 (80)
Heterotrophic bacteria	11,130,000 (801,395)	12,060,000 (1,293,505)	19,405,000 (1,596,427)	21,420,000 (1,357,985)
Coliphage	260 (62)	290 (64)	524 (26)	798 (69)**
Fixed flat plate				
E. coli	2260 (163)	2540 (139)	3930 (715)	3890 (288)
Total coliforms	4250 (143)	4430 (173)	5160 (468)	6130 (318)
C. perfringens (spore)	245 (34)	40 (16)***	975 (96)	1055 (86)
C. perfringens (vegetative)	590 (69)	635 (53)	1680 (86)	1775 (67)
Heterotrophic bacteria	34,230,000 (4,148,709)	30,005,000 (1,721,021)	8,970,000 (695,390)	10,570,000 (822,874)
Coliphage	1350 (133)	1640 (125)	1720 (339)	1550 (147)
Fixed grooved plate				
E. coli	148 (14)	141 (8)	1865 (119)	1975 (96)
Total coliforms	2110 (177)	1720 (278)	4500 (226)	4340 (434)
C. perfringens (spore)	1425 (94)	1885 (126)*	681 (52)	562 (42)**
C. perfringens (vegetative)	2595 (136)	3050 (333)	885 (111)	800 (44)
Heterotrophic bacteria	8,470,000 (1,024,700)	6,550,000 (1,148,840)	2,370,000 (168,689)	2,290,000 (287,306)
Coliphage	212 (18)	250 (17)*	1865 (216)	1737 (273)

[a] Values in parentheses are SEM (n = 10). Asterisks (*, **, and ***) indicate significant differences at the 0.05, 0.01, and 0.001 probability levels, respectively.

to the consistency of the non-diluted wastewater, during the 276 kPa runs it was often necessary to remove the nozzle to maintain proper pressure. As a result, these data are presented separately in table 3. In general, the pre- and post-sprinkler microorganism concentrations were similar and within the same order of magnitude. A statistical analysis of the data, however, revealed that the post-sprinkler concentration was statistically greater than the pre-sprinkler concentration in several instances. For example, at a pressure of 207 kPa with a rotating spray plate, the post-sprinkler *C. perfringens* spore concentration was about 2-fold greater ($p < 0.01$) than the pre-sprinkler concentration (table 2). Similarly, under the same test conditions, post-sprinkler *E. coli* and coliphage concentrations were found to be greater ($p < 0.01$). At a pressure of 138 kPa with use of a grooved spray plate, both the *C. perfringens* spore and coliphage concentrations were statistically greater ($p < 0.05$) in the post-sprinkler discharge (table 2). When the sprinkler pressure was increased to 276 kPa, total coliform and coliphage concentrations were found to be greater ($p < 0.01$). None of the post-sprinkler concentrations were found to be statistically greater at each of the pressures tested when a flat spray plate was used (table 3).

In only two instances were the post-sprinkler concentrations found to be statistically lower than the pre-sprinkler concentrations (table 2). At a sprinkler pressure of 138 kPa with a flat spray plate, the mean pre- and post-sprinkler *C. perfringens* spore concentrations were 245 and 40 CFU mL^{-1}, respectively. When a grooved spray plate was used at 207 kPa, the respective mean pre- and post-sprinkler *C. perfringens* spore concentrations were 681 and 562 CFU mL^{-1}. These results were unexpected, as unlike vegetative cells, clostridia spores are resistant to high pressures and other physical and chemical treatments (Paredes-Sabja et al., 2007). With respect to the rotating spray plate, no statistically significant post-sprinkler decreases occurred (tables 2 and 3).

Table 3. Mean culturable microorganism concentrations (CFU or PFU mL^{-1}) in pre- and post-sprinkler samples at a pressure setting of 276 kPa without a nozzle.[a]

Microorganism	Pre-Sprinkler	Post-Sprinkler
Rotating plate		
E. coli	47 (8)	46 (8)
Total coliforms	950 (118)	1040 (91)
C. perfringens (spore)	480 (39)	475 (56)
C. perfringens (vegetative)	725 (37)	798 (70)
Heterotrophic bacteria	3,550,000 (229,129)	3,560,000 (581,416)
Coliphage	1445 (127)	1540 (127)
Fixed flat plate		
E. coli	33 (6)	34 (7)
Total coliforms	1720 (181)	2330 (310)
C. perfringens (spore)	482 (37)	366 (29)
C. perfringens (vegetative)	1050 (125)	1130 (131)
Heterotrophic bacteria	4,290,000 (276,265)	4,030,000 (401,400)
Coliphage	1410 (92)	1890 (238)
Fixed grooved plate		
E. coli	1608 (69)	1716 (65)
Total coliforms	3510 (181)	4810 (249)***
C. perfringens (spore)	191 (25)	218 (10)
C. perfringens (vegetative)	533 (28)	520 (32)
Heterotrophic bacteria	14,080,000 (831,939)	16,884,167 (1,171,621)
Coliphage	136 (20)	358 (81)**

[a] Values in parentheses are SEM (n = 10). Asterisks (*, **, and ***) indicate significant difference at the 0.05, 0.01, and 0.001 probability levels, respectively.

In this study, we used fecal indicator organisms to investigate the effect of pressure and spray plate on post-sprinkler culturable concentrations. Indicator organisms are generally used in water quality studies and investigations into bioaero-

sol formation and transport since they are more abundant than target pathogens and are easily identified (Teltsch and Katzenelson, 1978; Brenner et al., 1988; Pianetti et al., 2004). While the use of indicator organisms provides a good approximation of sprinkler effects on microbial populations, effects on pathogens cannot be directly assessed from this data set, as they may respond differently to environmental stresses (Moriñigo et al., 1990; Harwood et al., 2005). Although there was no clear trend between the post-sprinkler microorganism concentrations and the sprinkler configuration, the results suggest that spray irrigation influences viable microorganism concentrations in wastewater in some cases.

In cases where the post-sprinkler concentrations were found to be lower (*Clostridium* spores only), it is likely that the integrity of the cellular membranes was disrupted due to the physical force of the microorganisms hitting the spray plate or by the sudden decrease in pressure as they were discharged from the nozzle. Shear stress in particular has been shown to be responsible for the inactivation of viral particles (Segura et al., 2006; Peixoto et al., 2007), although larger viral particles have been shown to be relatively unaffected (Michalsky et al., 2008). One must also consider the fact that these conditions could have transformed the microorganisms into a viable non-culturable condition (Bogosian and Bourneuf, 2001). When culture-based techniques are used to quantify microorganisms, those in a viable but non-culturable condition can cause underestimation of the actual risk of exposure and infection (Jones et al., 1991; Colwell et al., 1996). Use of alternative techniques (e.g., molecular-based techniques) can be employed to enhance the detection of non-culturable microorganisms, although some of these techniques cannot differentiate between viable and non-viable organisms (Dungan and Leytem, 2009).

Increases in the post-sprinkler concentration of vegetative cells, spores, and coliphages may be attributable to the disruption of microbial aggregates (e.g., via shear stress) during release of wastewater from the nozzle and impaction on the spray plate. While disruption of aggregates does not increase the total number of microorganisms per unit volume of wastewater, it increases the number of individual particles. This is an important consideration when using a dispersion model to predict downwind concentrations, as the actual number of microorganisms available for aerosolization could be substantially greater than the values determined in the wastewater prior to spray irrigation. During wastewater spray irrigation studies conducted by the U.S. EPA (1982), I was developed to account for the loss of microorganism viability during the spray process. In some cases, I was greater than 1, suggesting that wastewater aggregates were being broken apart during the aerosolization process. Since I was used as a catch-all for all undefined processes that influence the viable microorganism source strength (i.e., organisms s^{-1}), no efforts were made to define separate values for individual component of I. Our results indicate that the physical stresses associated with the spraying process can also influence microorganism viability prior to aerosolization, which should be accounted for in dispersion models.

Conclusions

The results from this study, while not producing any clear trends, indicate that spraying dairy wastewaters can result in both increases and decreases in post-sprinkler microorganism concentrations. However, the data do not support our hypothesis that increases in sprinkler pressure result in decreases in microorganism survivability. In most cases where a statistical difference was noted between pre- and post-sprinkler concentrations, an increase most often occurred. The concentration increase can be attributed to the disruption of microbial aggregates during the spraying process, whereas concentration decreases were likely a result of microorganism death. It should be noted that both disruption and inactivation processes may be a result of the same shear stresses and may occur simultaneously.

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