SURVIVAL OF FECAL BACTERIA IN DAIRY COW MANURE

L. Wang, K. R. Mankin, G. L. Marchin

ABSTRACT. *Bacterial pollution of water is impacted to a great extent by the ability of bacteria to survive in manure following excretion. We investigated the effects of environmental temperature (4˚C, 27˚C, and 41˚C) and manure moisture content (30%, 55%, and 83%) on the survival and release of indicator bacteria in dairy cow manure. Fresh manure samples of about 60 g were packed to 12 mm depth in polystyrene dishes and held at controlled temperatures and moisture contents for up to 103 days. Supernatant from a distilled−water extraction was enumerated for fecal bacteria (fecal coliforms,* Escherichia coli, *and fecal streptococci) by the membrane filtration method. Bacterial populations increased as much as 2.5 log_{10} (over 300×) in the three days following excretion. Temperature had a significant overall effect on survival of all three fecal bacteria, whereas moisture content produced a consistent effect on fecal streptococci survival only. Fecal streptococci showed no significant die−off at any temperature or moisture content studied. In contrast, no measurable* E. coli *or fecal coliforms were found in supernatant water samples from the 41˚C treatment after day 35.* E. coli *and fecal coliform populations for the 4˚C treatment at lower moisture content (30% and 55%) conditions were close to the detection limits after five weeks, but significant numbers of* E. coli *(2.34 × 10^4 cfu g^{-1} wet manure) and fecal coliforms (3.84 × 10^4 cfu g^{-1} wet manure) remained for the 4˚C treatment at 83% moisture content after 103 days. First−order die−off rate coefficients for* E. coli *were found to be appropriate after day 3 for about a 3−week period and averaged 0.11 d^{-1} at 4˚C, 0.20 d^{-1} at 27˚C, and 0.32 d^{-1} at 41˚C. Results from this study suggest that barnyard, feedlot, and manure management practices that detain manure at higher temperatures (e.g., 41˚C) will decrease the* E. coli *and fecal coliform populations but not those of fecal streptococci. Coliform bacterial populations tested remained viable for long periods (>3 months) particularly at moderate temperature (27˚C) for any moisture level, and streptococci survived under all conditions studied.*

Keywords. *Moisture, Pathogens, Temperature, Water quality.*

Livestock feedlots are major contributors of fecal pollution to surface water (Crane et al., 1983). Cow manure deposited on rangelands or farmlands also release fecal microbes that can be carried by runoff to surface water or through the soil matrix to groundwater. There is little data, however, on survival and release of water−extractable microbes in manure exposed to various environments.

The use of indicator organisms as surrogates for pathogenic fecal organisms in both fate and transport studies is common and reasonable. Research by Ogden et al. (2001) and Mubiru et al. (2000) showed that both pathogenic and non−pathogenic *Escherichia coli* strains have similar survival patterns in soil. Ogden et al. (2001) suggested that some non−pathogenic strains survive even longer than their pathogenic counterparts. Pundsack et al. (2001) found that the fecal coliform removal patterns in wastewater reflected those of *Salmonella* in both summer and winter seasons. Efforts have been made to develop practical yet reliable technologies for directly detecting pathogenic microorganisms. However, fecal bacteria, such as fecal coliforms, *E. coli*, and fecal streptococci, are still used for fecal contamination indication (e.g., Tian et al., 2002) and for treatment system evaluation (e.g., Pundsack et al., 2001).

Much research has focused on fecal bacteria survival in soil. However, manure provides a different biological and physico−chemical environment to bacteria. Kudva et al. (1998) found that *E. coli* O157:H7 survived longer in manure than in soil. Thelin and Gifford (1983) showed that bacterial survival and release were affected by the duration of manure exposure to the environment. Kress and Gifford (1984) confirmed this and found that 100−day−old cattle manure still produced fecal coliform counts as high as 4.2 × 10^3 colony forming units (cfu) per 100 mL.

Models with different levels of complexity have been proposed to describe fecal bacteria die−off in manure, soil, water, and other media under different environmental conditions (e.g., Crane et al., 1980; Entry et al., 2000a, 2000b). Among these, a simple first−order model is the most common (Reddy et al., 1981; Crane and Moore, 1986; Himathongkham et al., 1999):

$$M_t = M_0 \exp(-kt) \quad (1)$$

where M_t is the microbial concentration at time t, M_0 is the initial microbial concentration, t is time, and k is the first−order rate coefficient for the net die−off of bacteria, which is a function of temperature and typically described using the Arrhenius equation:

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$$k_{T2} = k_{T1}\,\theta\,\exp\,(T_2 - T_1) \qquad (2)$$

where T_1 and T_2 are temperatures (˚C), k_{T2} is the die−off rate adjusted to temperature T_2, k_{T1} is the die−off rate measured at temperature T_1, and θ is the temperature−correction coefficient. As temperature increased, however, Howell et al. (1996) noted that fecal bacteria mortality became increasingly nonlinear. Zhai et al. (1995) and Mubiru et al. (2000) used a two−stage first−order function (eq. 1 with two different M_0 and k coefficients) to describe *E. coli* mortality rates.

As suggested by equation 2, temperature is a key environmental factor that has been related to die−off rate. *E. coli* survived in soil under controlled conditions for a shorter period of time when held at 37˚C than at 5˚C, 10˚C, or 20˚C (Sjogren, 1994). Kudva et al. (1998) reported longer survival times for *E. coli* O157:H7 in manure at −20˚C, 4˚C, and 23˚C compared to 45˚C and 70˚C. Wang et al. (1996) also observed that *E. coli* O157:H7 survival in feces decreased with increasing temperature (70 days at 5˚C to 56 days at 22˚C and 49 days at 37˚C). Similarly, Himathongkham et al. (1999) reported more rapid die−off of *E. coli* O157:H7 in cow manure or slurry in an environment of 37˚C and 30% relative humidity as compared with either 20˚C and 50% or 4˚C and 73%.

Moisture content of manure or soil has also been associated with changes in fecal bacteria die−off rates. Wang et al. (1996) suggested that manure dehydration might contribute to die−off of *E. coli* O157:H7. The maximum survival rate of *Streptococcus faecalis* was found when soil was saturated, whereas the minimum survival period was found in air−dry soil (Kibbey et al., 1978). Sjogren (1994) also found a lower *E. coli* die−off rate in saturated Webb sandy loam than at 15% soil saturation at all temperatures tested (5˚C, 10˚C, 20˚C, and 37˚C). However, Sjogren (1994) found a slightly higher die−off rate under saturated soil conditions at 10˚C for Richmond sandy loam. Mubiru et al. (2000) observed a similar phenomenon and surmised that it was the matrix potential of individual soils that played a significant role when the soil moisture content was the same. Nevertheless, no studies appear to have isolated the effect of the moisture content on survival of fecal bacteria.

The goal of this study was to assess the potential for fecal bacteria contamination of surface runoff from exposed dairy−cow manure at different ages. Specifically, the objectives were to evaluate the survival and release of indicator bacteria (fecal coliforms, *E. coli*, and fecal streptococci) over time in dairy cow manure over a range of environmental temperatures and manure moistures that span typical field conditions and to assess the suitability of first−order kinetics for describing bacterial die−off under these conditions.

Materials and Methods

Manure Samples

Fresh dairy cow manure samples (about 3 to 4 kg total) were collected after morning lactation and before 9:00 a.m. on 27 July and 4 September 2001 directly from cow recta. Cow diet consisted of a mixture of hay, silage, corn, soybean meal, and other mineral supplements. The July trial (trial 1) used a mixture of manure from three cows, whereas the September trial (trial 2) used a mixture from four cows.

Wet−basis moisture content was determined gravimetrically at the beginning and end of each trial using three replicate 2 g manure samples for each treatment. Samples were dried at 103˚C to 105˚C until equilibrium, cooled to room temperature, and weighed. No significant weight loss of dry matter (<0.98%) in manure samples was observed at the end of each trial. The sample taken on July 27 had 83.9% average moisture, and the sample taken on September 4 had 82.7% average moisture. These values are similar to those reported by Reddy et al. (1981) and ASAE (*ASAE Standards*, 2002).

Fresh manure was distributed into 36 polystyrene dishes (100 mm dia. by 25 mm depth), with 60 ±1 g (trial 1) or 57 ±1 g (trial 2) in each dish. The dish was tapped on a flat surface to get a level manure depth of 12 mm. Four dishes were randomly assigned to each of nine treatments.

Experimental Design and Treatment Conditions

The experiment was arranged in a 2 × 3 factorial design with four replicates. Manure samples were treated at three temperature levels (mean ±range), i.e., 4˚C ±1˚C (refrigerator), 27˚C ±4˚C (room temperature, within a 189 L high−density polyethylene [HDPE] container), and 41˚C ±4˚C (oven), and three moisture−content (MC) levels (30% ±5% MC, 55% ±5% MC, and 83% ±5% MC). Evaluating the effects of moisture content on bacterial populations at other than "as−excreted" moisture levels is inherently challenging. The experimental design may dry the media to the target level rapidly, using an artificial environment, or allow moisture content to decrease gradually under ambient conditions; either approach risks non−treatment−related population responses during the transition. This study used the latter approach to avoid the potential shock to bacteria associated with rapid environmental change and better simulate the transition in moisture content experienced in the field. Manure samples for the 30% MC and 55% MC treatments were left uncovered until they reached the target weight, except for samples held at 41˚C, which were left covered for most of the dry−down period (fig. 1). Samples kept at 4˚C and 27˚C took about 9 days to reach 55% MC and 13 days to reach 30% MC. At 41˚C, samples were covered for 6 days and then left uncovered, which allowed them to reach 55% MC at day 7 and 30% MC at day 9. Samples were weighed twice a day. Distilled water was sprayed directly onto the manure as needed to replace evaporative losses and maintain moisture content in the desired range.

Trial 1 lasted 32 days. Bacterial extractions were prepared at days 3 (83% MC only), 7 (83% MC only), 19, and 32. Manure samples at moisture levels of 30% and 55% had not reached target moisture levels by days 3 and 7 and were not tested on these two days. Trial 2 was run for 103 days, and bacterial extractions were prepared at days 3 (83% MC only), 10 (83% MC only), 20, 35, 71, and 103.

Water Sample Preparation

Manure samples of 0.2 cm^3 (150 to 500 mg wet manure, similar to Himathongkham et al., 1999) were collected from each dish using two subsamples from a 5 mm diameter stainless steel tube (for the 30% and 55% MC treatments) or using plastic knife and spoon (83% MC treatment). Manure samples in aluminum dishes were weighed at room temperature by an analytical balance (0.1 mg accuracy). Distilled water (10 mL) was added into each aluminum dish, and the mixture was transferred into a 1000 mL beaker where the

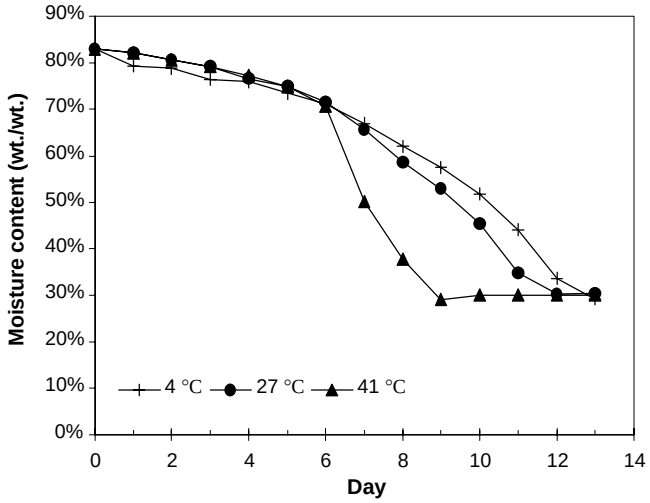

Figure 1. Typical changes in manure moisture content during the first two weeks.

manure particles were smashed with a sanitized bamboo stick. The aluminum dish was rinsed with distilled water at least three times to minimize manure remaining in the dish. A total of 500 ±10 mL of distilled water was added to each beaker and stirred for about 1 min. The mixture was allowed to settle for 30 min, and then 50 mL of supernatant was collected for analysis. Bacterial extractions were performed within 2 h of sampling and were plated for bacteria within 6 h of sampling.

All implements were disinfected in HCl solution for at least 24 h, rinsed under running tap water until pH went back to normal (6.8 to 7.4), rinsed again with distilled water, and dried at room temperature.

Indicator Bacteria Enumeration

The membrane−filtration method was used for bacterial enumeration of fecal coliforms (Part 9222D, APHA, 1998), *E. coli* (Part 9222G, APHA, 1998), and fecal streptococci (Hagedorn et al., 1999). Samples of 10 mL to 10 µL were initially plated to measure the range of organism concentration. Afterward, serial 1:10 dilutions were made in physiological saline solution.

A 20 to 30 mL volume of sample and rinse water was filtered through 0.45 µm gridded sterile membrane, and the membrane was placed into specific media for each type of bacteria: mFC (Difco, Detroit, Mich.) for fecal coliforms, and KF (Difco, Detroit, Mich.) for fecal streptococci. The mFC plates were incubated at 44.5˚C ±0.2˚C for 24 h, and the KF plates were incubated at 37˚C for 48 h. After fecal coliforms were counted, the filters were transferred to MUG media (4−methylumbelliferyl−β−D glucuronide, Difco, Detroit, Mich.) and incubated for 4 to 6 h at 37˚C for *E. coli* enumeration. The bacterial counts on each plate within the recommended counting range (20 to 60 cfu) were converted to cfu per gram of manure sample (at a given moisture content). If the bacterial counts were not within the recommended counting range, then the number of cfu was estimated by the method recommended in the *Standard Methods* (Part 9222B, APHA, 1998). The detection limit for these methods was approximately 10 cfu/100 mL, which translates into 96 ± 16 cfu g^{-1} for 83% MC treatments, 397 ± 35 cfu g^{-1} for 55% MC, and 405 ± 34 cfu g^{-1} for 30% MC, where standard deviations are based on variability of actual manure sample masses. For analysis, a value of 80% of the detection limit was assumed for those samples that showed zero colony counts.

Data Analysis

Experimental data were analyzed using a commercial spreadsheet to calculate treatment means, regression coefficients, and standard deviations. An analysis of variance for a completely randomized design with repeated measurements across dates was used to determine if overall treatment effects existed. The treatment effects were assessed based on microbial counts normalized by manure dry weight. Specific comparisons among treatment means at each date were achieved with the least−significant difference (LSD) test. The Mixed and General Linear Model procedures of SAS (SAS, 2000) were used.

Results and Discussion

Initial Bacteria Concentrations

E. coli populations extracted from fresh cow manure were fairly consistent between trials (6.55×10^6 and 7.60×10^6 cfu g^{-1} dry manure) and had the least within−trial variability (CV = 0.19) compared with fecal coliforms and fecal streptococci. In both trials, as−excreted concentrations of fecal coliforms (1.20×10^7 and 1.65×10^7 cfu g^{-1} dry manure) were only slightly lower than that found by Doyle et al. (1975) (4.67×10^7 cfu g^{-1}), higher than those reported by Reddy et al. (1981) (1.38×10^6 cfu g^{-1}), and similar to the mean value in *ASAE Standards* (2002) (1.33×10^7 cfu g^{-1}). Populations of fecal streptococci had two orders of magnitude difference between trials, ranging from 9.31×10^6 cfu g^{-1} dry manure in trial 1 to 9.52×10^4 cfu g^{-1} dry manure in trial 2; both were lower than that reported by Doyle et al. (1975) (2.70×10^7 cfu g^{-1}) and by *ASAE Standards* (2002) (7.67×10^7 cfu g^{-1}). Trial 1 was comparable, however, to that compiled by Reddy et al. (1981) (7.78×10^6 cfu g^{-1}). Therefore, the as−excreted bacterial populations obtained using distilled−water extraction in this study fell within the characteristic ranges for dairy cow manure. It should be noted that no measurement was made of the bacterial populations remaining in the manure after extraction.

Standard extraction of bacteria from soil often uses dilute saline solution to minimize the "osmotic shock" that bacteria may experience upon submersion in water, and consequently to maximize colony survival during extraction. Several studies obtained bacteria extraction by mixing manure samples with a 0.15 N sodium chloride solution (Wang et al., 1996; Kudva et al., 1998; Bolton et al., 1999; Himathongkham et al., 1999). Municipal water treated with chemicals to remove chlorine (Thelin and Gifford, 1983; Kress and Gifford, 1984; Larsen et al., 1994) or reverse−osmosis treated water (Brush, 1997; Gagliardi and Karns, 2000) have also been used. In this study, distilled−water extraction was used to represent rainwater conditions and allow more reasonable extension to field bacterial release conditions. The numbers of *E. coli* and fecal streptococci extracted from fresh cow manure in the current work were comparable with values reported in the literature cited above.

Table 1. Means and main treatment effects of *E. coli*, fecal coliforms, and fecal streptococci concentrations with time. Initial concentrations (all values in $\log_{10}$ cfu g^{-1} dry manure) were 6.82 (trial 1) and 6.88 (trial 2) for *E. coli*, 7.03 (trial 1) and 7.20 (trial 2) for fecal coliforms, and 6.97 (trial 1) and 4.98 (trial 2) for fecal streptococci.

			Day, Trial 1		Day, Trial 2			
Treatment		Level	19	32	20	35	71	103
E. coli ($\log_{10}$ cfu g^{-1} dry manure)								
	LSD[a]		0.39	0.67	0.67	0.42	0.27	0.46
	Moisture	30%	4.15 b	5.21 a	4.14 a	4.86 ab	4.03 a	3.19 b
		55%	3.76 b	5.02 a	4.20 a	5.20 a	3.97 a	3.01 b
		83%	4.77 a	5.42 a	4.64 a	4.45 b	3.64 b	3.68 a
	Temperature	41°C	3.23 c	3.33 c	3.13 b	2.72 c	2.69 c	2.69 b
		27°C	5.50 a	6.75 a	6.34 a	7.79 a	5.39 a	3.63 a
		4°C	3.95 b	5.58 b	3.52 b	4.00 b	3.56 b	3.55 a
Fecal coliforms ($\log_{10}$ cfu g^{-1} dry manure)								
	LSD[a]		0.44	0.75	0.82	0.38	0.32	0.36
	Moisture	30%	4.33 b	5.76 a	4.47 a	4.90 b	4.21 a	3.41 b
		55%	3.86 c	5.42 a	4.87 a	5.42 a	4.09 a	3.11 b
		83%	5.13 a	5.51 a	4.82 a	4.52 b	3.92 a	3.78 a
	Temperature	41°C	3.31 c	3.51 c	3.27 c	2.77 c	2.69 c	2.65 c
		27°C	5.83 a	6.97 a	6.41 a	7.92 a	5.74 a	4.03 a
		4°C	4.17 b	6.19 b	4.48 b	4.15 b	3.78 b	3.63 b
Fecal streptococci ($\log_{10}$ cfu g^{-1} dry manure)								
	LSD[a]		0.26	0.32	0.40	0.49	0.35	0.32
	Moisture	30%	4.32 c	6.09 c	5.20 a	5.19 b	5.13 c	5.00 b
		55%	4.88 b	6.55 b	5.43 a	5.82 b	5.81 b	5.70 a
		83%	6.06 a	7.13 a	5.45 a	6.30 a	5.99 a	5.88 a
	Temperature	41°C	4.63 b	5.34 c	4.80 b	4.66 c	4.99 c	5.19 b
		27°C	5.93 a	6.12 b	6.59 a	6.82 a	6.05 a	5.49 b
		4°C	4.70 b	8.30 a	4.69 b	5.83 b	5.77 a	5.89 a

[a] LSD (least−significant difference) values. Means in the same column for a given treatment with the same letter indicate no significant difference at $\alpha = 0.05$.

Temperature and Moisture Effects

Over the 103−day period, temperature had a significant effect on fecal coliforms and *E. coli* survival. On all sampling dates, the population of *E. coli* was highest at 27˚C followed, in order, by 4˚C and 41˚C (table 1). These differences were significant for fecal coliforms in all cases. Populations of *E. coli* at 27˚C were significantly greater than those observed at 41˚C in all cases. The highest population was found at 27˚C, in agreement with observations on *E. coli* O157:H7 by Conner and Kotrola (1995); they found the highest population at 25˚C during 56 days of treatment.

Moisture−content effects on *E. coli* and fecal coliform survival were variable (table 1). *E. coli* populations were highest for the 83% MC treatment for all cases except 35 and 71 days in trial 2. No significant differences were found between 30% and 55% MC treatments on all sampling dates. Fecal coliform populations followed similar trends.

Like fecal coliforms and *E. coli*, the highest fecal streptococci populations were found at 27˚C (except at day 103, trial 2) and the lowest at 41˚C (table 1). Fecal streptococci populations on all sampling dates were highest at 83% MC followed, in order, by 55% MC and 30% MC, with 83% MC significantly greater than 30% MC in all cases expect day 20, trial 2.

Interaction between temperature and moisture content was observed in trial 2, as was the case with the study by Sjogren (1994). At 4˚C, die−off of *E. coli* over the 103−day study period was least (1.78 $\log_{10}$ reduction) at the highest manure moisture content, 83% MC (fig. 2f). At 27˚C, however, the die−off increased with moisture contents (*E. coli* reductions over the 103−day period were 2.05 $\log_{10}$ reduction at 30% MC, 3.12 $\log_{10}$ reduction at 55% MC, and 3.52 $\log_{10}$ reduction at 83% MC, fig. 2). Previous conclusions that survival was highest at higher moisture contents (Wang et al., 1996; Himathongkham et al., 1999) were based on only lower temperatures and did not use an adequate experimental design to evaluate the temperature interaction.

Bacterial Growth Period

Preliminary work (not shown) and results of other studies indicated that bacterial populations in manure often increased during the first week following excretion and peaked after 2 to 3 days, as discussed below. Over this period, manure from the 30% and 55% MC treatments had not yet reached stable moisture contents; thus, only the 83% MC samples were analyzed for bacterial growth in the first 10 days. *E. coli* populations at 27˚C increased by 1.95 $\log_{10}$ in trial 1 (fig. 2e) and 2.25 $\log_{10}$ in trial 2 (fig. 2f) at day 3. *E. coli* populations at 27˚C remained higher than the initial population even at day 7 in trial 1 and day 10 in trial 2. At 4˚C and 41˚C, *E. coli* populations increased in trial 1 but not in trial 2. Fecal coliforms showed the same survival pattern as *E. coli* (data not shown). Fecal streptococci growth was observed at day 3 for all treatments, except at 4˚C in trial 2 (figs. 3e and 3f). Population increase at 41˚C was almost negligible. Fecal streptococci showed a growth pattern on day 3 (27˚C > 41˚C > 4˚C) that was similar to that of *E. coli* (figs. 2f and 3f).

Howell et al. (1996) reported that fecal coliforms and fecal streptococci increased for the first 3 days after dairy manure

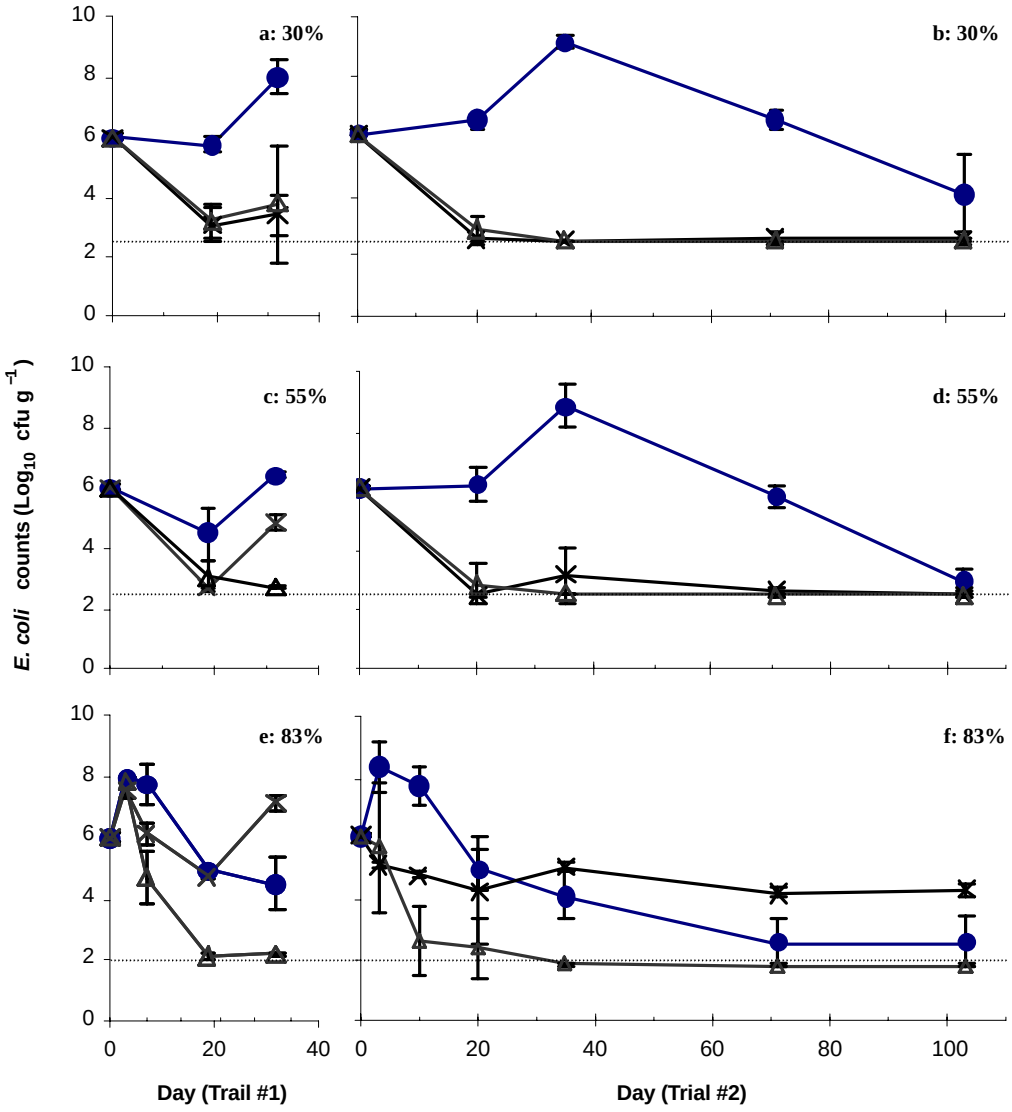

Figure 2. *E. coli* counts (mean ±SD) per wet manure mass from water extracts of manure at 4˚C (× shapes), 27˚C (black circles), and 41˚C (triangles) and at moisture contents of 30% (a, b), 55% (c, d), and 83% (e, f) for trial 1 (a, c, e) and trial 2 (b, d, f). Dotted lines indicate detection limits (96 ± 16 cfu g^{-1} for 83% MC, 397 ±35 cfu g^{-1} for 55% MC, and 405 ±34 cfu g^{-1} for 30% MC).

was added to sediment at 25˚C or 35˚C. Wang et al. (1996) reported a 2 $\log_{10}$ increase of inoculated *E. coli* O157:H7 population in manure after 2 days at 37˚C. Conner and Kotrola (1995) reported an even greater increase at 25˚C (2 to 4 $\log_{10}$). Fecal coliforms released from manure in a study by Thelin and Gifford (1983) also peaked at day 2. They suggested that the population of fecal coliforms in manure was in an exponential growth phase upon excretion. Once outside the gut and subject to growth resource depletion and adverse environmental conditions, the population goes through a growth−rate decline phase (about 1 day in their study), a stationary growth−rate phase (maximum population) of about 2 days, and then a death phase beyond 30 days. Our results indicated a decline phase for *E. coli* extending about 3 days, followed by a 20− to 35−day death phase at 41˚C, a 7− to 35−day stationary phase at 27˚C, and an intermediate response at 4˚C that interacted with moisture content (fig. 2).

Bacterial Die−Off Period

Overall reductions of *E. coli* after 103 days at 4˚C were 1.78 $\log_{10}$ at 83% MC (fig. 2f), 3.61 $\log_{10}$ at 55% MC (fig. 2d), and 3.56 $\log_{10}$ at 30% MC (fig. 2b). At 27˚C, the highest reduction was found at 83% MC (3.52 $\log_{10}$, fig. 2f), the lowest rate at 30% MC (2.05 $\log_{10}$, fig. 2b), and the median at 55% MC (3.12 $\log_{10}$, fig. 2d). This was in contrast with observations by other researchers (Sjogren, 1994; Wang et al., 1996; Himathongkham et al., 1999); all of them suggested that higher moisture content related to better survival, although none of these studies were designed to isolate the effect of moisture content. Higher moisture contents almost always were associated with low temperatures, as was the case reported by Himathongkham et al. (1999). Fecal streptococci populations were the lowest at 41˚C for higher moisture treatments and were the same or greater than the initial counts after 103 days for all treatments (fig. 3), except for trial 2 at 30% MC (fig. 3b). The highest counts over each study period were found at 4˚C for all treatments in both trials. Under most conditions, a greater fraction of fecal streptococci survived over the study period than *E. coli*.

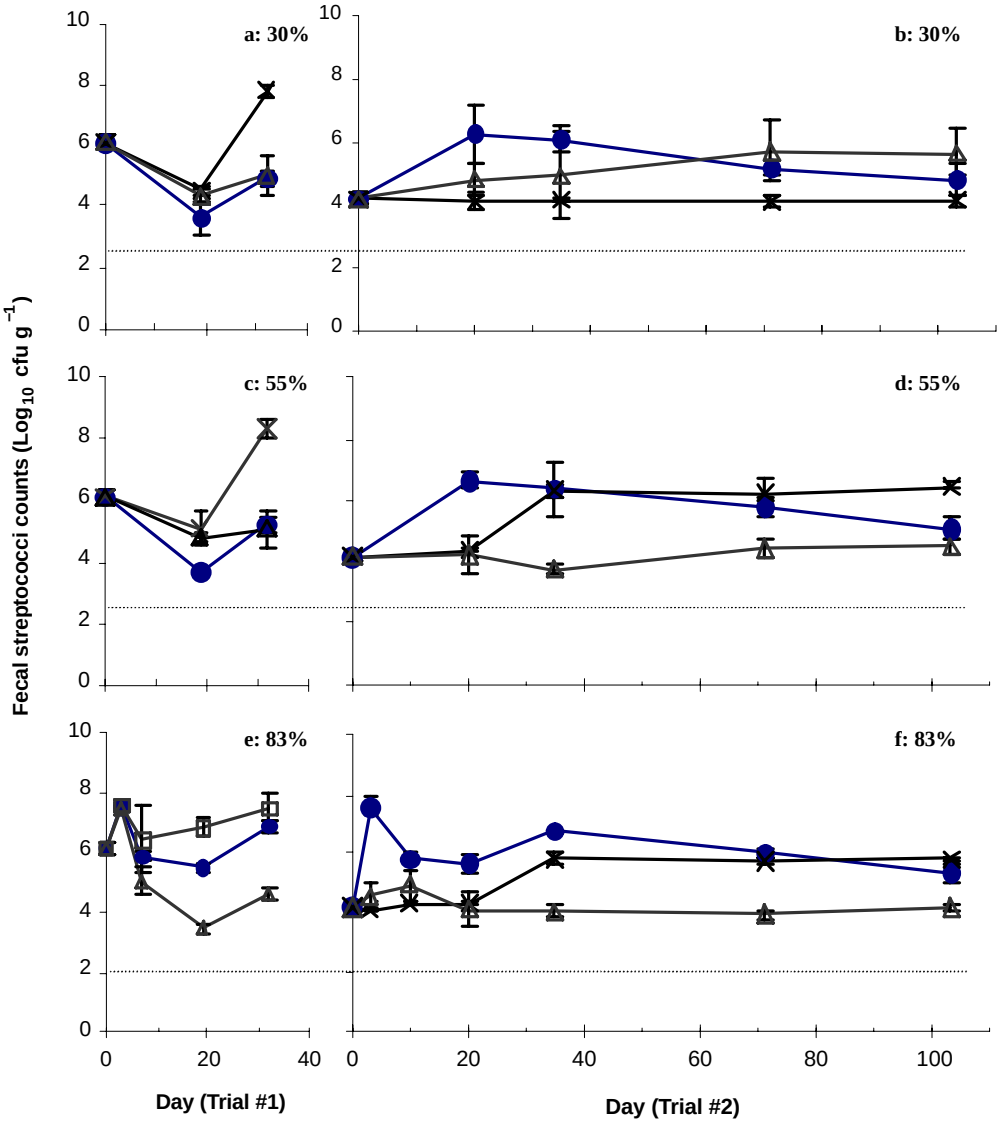

Figure 3. Fecal streptococci counts (mean ±SD) per wet manure mass from water extracts of manure at 4˚C (× shapes), 27˚C (black circles), and 41˚C (triangles) and at moisture contents of 30% (a, b), 55% (c, d), and 83% (e, f) for trial 1 (a, c, e) and trial 2 (b, d, f). Dotted lines indicate detection limits (96 ±16 cfu g^{-1} for 83% MC, 397 ±35 cfu g^{-1} for 55% MC, and 405 ±34 cfu g^{-1} for 30% MC).

E. coli Die−Off Kinetics

An exponential reduction of *E. coli* populations was observed for samples with moisture contents of 83% after day 3 for about a 3−week period (fig. 2). Like Mubiru et al. (2000), we found that a single−stage first−order mortality rate model did not adequately describe fecal bacteria die−off for the whole study period. From day 3 to day 19 (trial 1) or 20 (trial 2), the first−order model could be applied (fig. 4). The die−off rate coefficients of *E. coli* increased with increased temperature, averaging 0.11 d^{-1} at 4˚C, 0.20 d^{-1} at 27˚C, and 0.32 d^{-1} at 41˚C. The most consistent coefficients were derived for *E. coli* at 27˚C, with k = 0.197 d^{-1} (R^2 = 0.97) in trial 1 and k = 0.204 d^{-1} (R^2 = 0.94) in trial 2. The die−off rate calculated from trial 2 at 41˚C (k = 0.184 d^{-1}, fig. 3f) was less than that at 27˚C (k = 0.204 d^{-1}, fig. 2d) because most samples gave no *E. coli* counts at day 9 and after. Therefore, the die−off rate from trial 2 at 41˚C was not used in averaging. Compared with values cited by Crane and Moore (1986) (0.102 to 0.287 d^{-1}), slightly higher die−off rates (0.11 to 0.32 d^{-1}) were found in this study. It is likely that the wider range of our temperature treatments contributed to this difference; their data were obtained under a relatively constant temperature (in a laboratory in February). In addition, because *E. coli* populations at day 3 were generally greater than fresh manure (day 0), rate coefficients calculated in this study using data at day 3 for the initial population would be greater as well.

Using the simplified form of the Arrhenius equation (eq. 2), as suggested by both Crane and Moore (1986) and Reddy et al. (1981), θ was calculated as 1.026 for temperature between 4˚C to 27˚C, and 1.034 for temperature between 27˚C to 41˚C, all within the range (1.07 ±0.05) given by Reddy et al. (1981) in the temperature range at which most of the biological reactions occur. Using θ of 1.034 and k_{27} of 0.20 d^{-1}, the die−off rate at 37˚C would be k_{37} = 0.279 d^{-1}, which was the same as the k_{37} reported for *E. coli* O157:H7 by Himathongkham et al. (1999) at a relative humidity of 30%. This suggests that these die−off rates may be applicable for pathogenic *E. coli* O157:H7 as well. Although the rates were determined only for 83% MC in this

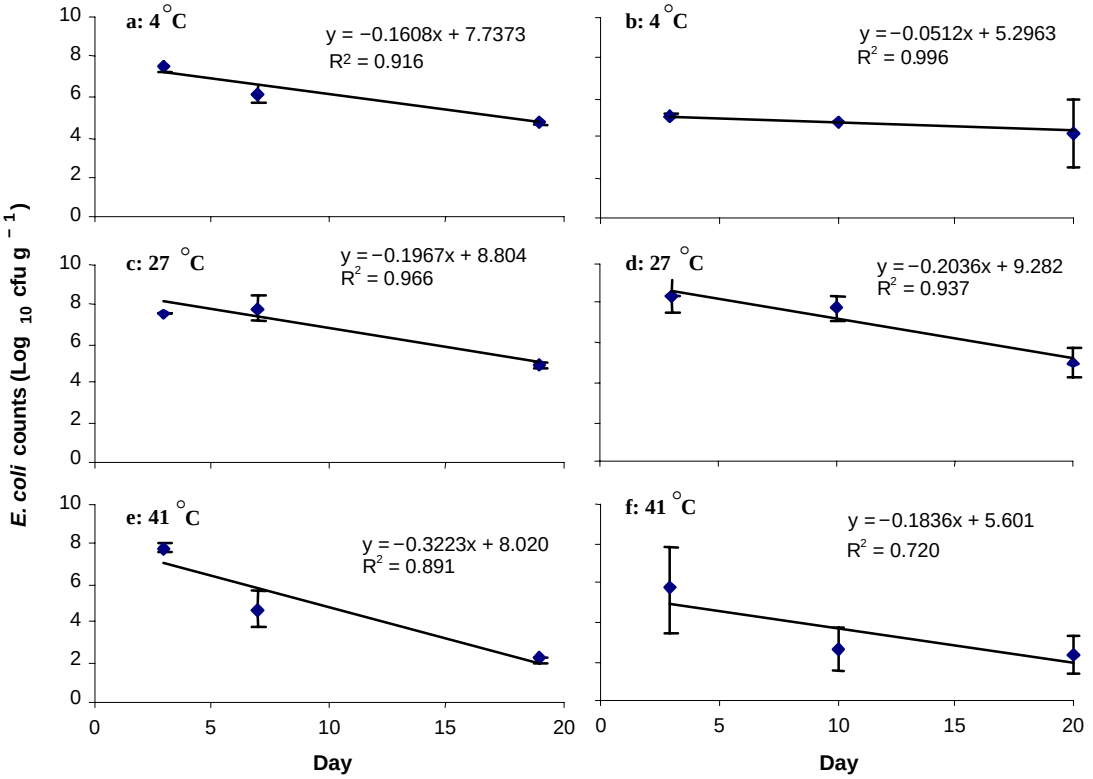

Figure 4. Regression of *E. coli* extracted from manure at 83% moisture content and 4˚C (a, b), 27˚C (c, d), and 41˚C (e, f) for trial 1 (a, c, e) and trial 2 (b, d, f).

study, the results may be more widely applicable because moisture content within the first 3 weeks did not have a significant effect on *E. coli* die−off.

CONCLUSIONS

Populations of almost all bacteria at 83% moisture content increased during the first three days. This suggests a continuation of bacterial growth dynamics from intestinal conditions and indicates that as−excreted estimates of manure bacterial populations may underestimate populations available for contamination of surface runoff for several days after excretion.

Fecal streptococci populations were consistently ranked by moisture content (83% > 55% > 30%) and were greatest at 41˚C in most cases. Notably, after 103 days, no treatments had fecal streptococci populations below initial levels.

Temperature significantly affected die−off of *E. coli* and fecal coliforms, with the highest reductions at 41˚C and the lowest at 27˚C. Survivals of *E. coli* and fecal coliforms were generally highest at 83% moisture content, although the effect of the moisture treatment was variable. These results suggest that barnyard, feedlot, and manure management practices that allow manure to be exposed to high temperatures and, in some cases, lower moisture contents within reasonable environmental ranges will decrease *E. coli* or fecal coliforms populations. However, detectable populations were present even after 103 days for most treatments.

First−order kinetics adequately described *E. coli* die−off after the initial growth period for about the next three weeks only, with rate coefficients that increased with temperature. Clearly, more complex models are necessary to capture the bacterial growth and die−off dynamics for longer periods. However, for a reasonable period after excretion, a simple first−order model may capture changes in bacterial populations in manure, which would allow population estimates.

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