Fate of Coliform Bacteria in Composted Beef Cattle Feedlot Manure

Francis J. Larney,* L. Jay Yanke, James J. Miller, and Tim A. McAllister

ABSTRACT

The link between livestock production, manure management, and human health has received much public attention in recent years. Composting is often promoted as a means of sanitizing manure to ensure that pathogenic bacteria are not spread to a wider environment during land application. In a two-year study (1998 and 1999) in southern Alberta, we examined the fate of coliform bacteria during windrow composting of cattle (*Bos taurus*) manure from feedlot pens bedded with cereal straw or wood chips. Numbers of total coliforms (TC) and *Escherichia coli* declined as the composting period progressed. In 1998, TC levels (mean of both bedding types) were $\log_{10}$ 7.86 cells g^{-1} dry wt. for raw manure on Day 0, $\log_{10}$ 3.38 cells g^{-1} by Day 7, and $\log_{10}$ 1.69 cells g^{-1} by Day 14. More than 99.9% of TC and *E. coli* was eliminated in the first 7 d when average windrow temperatures ranged from 33.5 to 41.5°C. The type of bedding did not influence the numbers of TC or *E. coli*. Dessication probably played a minor role in coliform elimination, since water loss was low (<0.07 kg kg^{-1}) in the first 7 d of composting. However, total aerobic heterotroph populations remained high (>7.0 $\log_{10}$ CFU g^{-1} dry wt., where CFU is colony forming units) throughout the composting period, possibly causing an antagonistic effect. Land application of compost, with its nondetectable levels of *E. coli* compared with raw manure, should minimize environmental risk in areas of intensive livestock production.

THERE IS GROWING public concern about the link between livestock production and water contamination by pathogenic bacteria. This is especially true for land application of raw manure, which potentially spreads pathogens to a wider environment (Bach et al., 2002; Kudva et al., 1998; Pell, 1997). Entry and Farmer (2001) reported fecal coliform bacteria (which originate from the intestinal tracts of warm-blooded animals) in ground water flowing from an Idaho aquifer. Gagliardi and Karns (2000) demonstrated that if *Escherichia coli* reached soil, via manure spreading or runoff from a point source, it could survive, replicate, and move downward for up to two months, threatening nontarget environments.

Escherichia coli O157:H7 is one of the many strains of the bacterium *E. coli*. In Canada, *E. coli* O157:H7–contaminated water caused seven deaths and made more than 2000 people ill in Walkerton, Ontario in May 2000. The outbreak was linked to contamination of the town's water supply from land application of livestock manure on a nearby farm (O'Connor, 2002). Even land application of stockpiled manure poses a threat, since Kudva et al. (1998) reported that *E. coli* O157:H7 survived for more than one year in a nonaerated ovine manure pile that was exposed to environmental conditions.

A rapid increase in the number of intensive livestock operations in Alberta over the past decade has compelled many farmers to seek alternative methods to direct land application of raw manure. By 2002, the adoption of manure composting had increased to at least 12 feedlots in Lethbridge County, in southern Alberta. Composting allows transportation of nutrients (especially nitrogen and phosphorus) from high nutrient-loading areas and also reduces odor complaints at land application (Rynk, 1992). It eliminates the parasitic protozoa *Giardia* and *Cryptosporidium* (McAllister, unpublished data, 1999) and reduces weed seed viability (Larney and Blackshaw, 2003). The principal mode of disinfection during composting is based on time–temperature relationships that destroy pathogens, although antagonistic microorganisms and ammonia may also play a role (Epstein, 1997; Himathongkham and Riemann, 1999). For pathogen elimination during windrow composting of biosolids, temperatures should be maintained at >55°C for 15 d or longer (USEPA, 1992). During this period, the windrow should be turned a minimum of five times. Similar guidelines exist in Canada, which also state that final composts should contain <$\log_{10}$ 3 cells g^{-1} dry wt. of fecal coliforms (Canadian Council of Ministers of the Environment, 1996).

However, even though the elimination of pathogens by composting has been well documented (Déportes et al., 1998; Krogmann et al., 2002; Tiquia et al., 2002), composting regimes (time, temperature) required to achieve elimination of TC, *E. coli*, or other pathogens vary widely. Turner (2002) demonstrated inactivation of *E. coli* in farmyard manure, pig (*Sus scrofa*) feces, and cereal straw after only 2 h at 55°C. In contrast, Schleiff and Dorn (1997) reported that *E. coli* could be cultured from dry poultry (*Gallus gallus domesticus*) manure after 88 d of composting. Pathogen reduction with increasing time was shown by Himathongkham and Riemann (1999), who reported that while *E. coli* O157:H7 was able to grow for 2 d in fresh chicken manure at 20°C, with a resulting 1 to 2 $\log_{10}$ unit increase in colony forming units (CFU), increasing storage time to 6 d decreased populations by 3 to 4 $\log_{10}$ units. The effect of higher temperatures on reducing the time required for pathogen reduction was illustrated by Himathongkham et al. (1999), who reported a 10^5–fold reduction in *E. coli* O157:H7 after 105 d at 4°C or 45 d at 37°C in a laboratory incubation with cattle manure. Also, Droffner and Brinton (1995) found that *E. coli* survived for 59 d at 60°C, compared with only 9 d at 60 to 70°C in an inoculated laboratory food waste compost.

Agriculture and Agri-Food Canada, Research Centre, 5403 1st Ave. S., Lethbridge, Alberta, Canada T1J 4B1. Lethbridge Research Centre Contribution no. 38702070. Received 10 Sept. 2002. *Corresponding author (larney@agr.gc.ca).

Published in J. Environ. Qual. 32:1508–1515 (2003).

Abbreviations: CFU, colony forming units; MDL, minimum detection level; TAH, total aerobic heterotrophs; TC, total coliforms.

The mechanism for removal of pathogens during aerobic composting may not simply be a result of the thermal environment. Turner (2002) indicated that pathogen inactivation was not merely dependent on temperature but was also affected by water content and nature of the substrate. If incomplete inactivation occurred due to low temperatures, recovery and regrowth of the damaged pathogenic populations may be possible. In a study on composted dairy waste solids as recycled bedding, Mote et al. (1988) found that even though there was an initial decline or even a disappearance of TC due to composting, the bacteria reestablished in large numbers without reinocculation. Droffner et al. (1995) presented evidence for the survival of *E. coli* in active compost that indicated that, although classified as a mesophile, it had a mechanism for survival and perhaps replication at elevated temperatures (>60°C).

Although pathogen elimination is a recognized benefit of composting, the lack of definitive relationships between elimination and composting duration, substrates, and temperature conditions prompted a study on the fate of coliform bacteria during open-air windrow composting of beef feedlot manure in southern Alberta. Since there has been a recent increase in the use of byproducts from Alberta's forest industry (wood chips, shavings, sawdust) as alternative bedding to traditional cereal straw for feedlot cattle (McAllister et al., 1998), we compared manure from feedlot pens bedded with straw or wood chips. There is some evidence to suggest that wood products contain antimicrobial compounds that may inhibit bacterial levels (Allison and Anderson, 1951; Kudva et al., 1998).

This is the first study of its kind in the intensive feedlot area of southern Alberta, where manure management practices are coming under increased public and media scrutiny. It sought to quantify the duration of composting and accompanying windrow temperatures required to achieve coliform population reductions under typical field conditions.

MATERIALS AND METHODS

Windrow Establishment and Turning

The study was performed at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta during the summers of 1998 and 1999. Manure was removed with a loader and truck from feedlot pens, which had been bedded with barley (*Hordeum vulgare* L.) straw or wood chips, and deposited into windrows. The wood chips were a mixture of sawdust and bark peelings derived from 80% lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) and 20% white spruce [*Picea glauca* (Moench) Voss]. Windrows were established on 22 June 1998 and 20 July 1999 on a concrete pad in an open-sided roofed composting facility so that they were exposed to ambient air temperatures but not precipitation. There were two replicates of each bedding material for a total of four windrows, each on an east–west orientation. At establishment (Day 0) windrows were 10.6 to 11.4 m long, approximately 2.5 m wide at the base, and approximately 2 m high. Carbon to nitrogen ratios (dry combustion in an automated elemental C:N:S analyzer; Carlo Erba, Milan, Italy) were 19.0 for straw- and 24.7 for wood chip–bedded manure in 1998 and 14.4 for straw- and 23.6 for wood chip–bedded manure in 1999.

In 1998, the windrows were turned 16 times (Days 3, 7, 10, 14, 17, 21, 24, 28, 31, 38, 45, 52, 66, 73, 94, and 108 of composting) with a tractor-pull EarthSaver windrow turner (Fuel Harvesters Equipment, Midland, TX). This turning frequency was higher than at most commercial feedlots in southern Alberta (approximately five to seven times over a 90-d period). In 1999, the windrows were turned seven times (Days 7, 14, 21, 29, 42, 56, and 70), which was closer to the turning frequency at commercial feedlots. Turning was more frequent in the early stages of composting to stimulate thermophilic (>40°C) microbial activity and became less so as composting progressed and microbial activity diminished. After the active composting phase (turning) the material entered a "curing" phase (no turning) for a further 90 to 100 d until windrow temperature approached ambient.

Compost Sampling

Compost sampling for bacterial enumeration coincided with turning and sampling for chemical and physical properties (Larney et al., 2001). In 1998, windrows were sampled eight times: at establishment (Day 0) and just before turning on Days 7, 14, 21, 28, 45, 66, and 94. There were nine sampling times in 1999: at Day 0 and just before turning on Days 7, 14, 21, 28, 42, 56, and 70, as well as Day 91 (no turning event).

In 1998, the sampling protocol involved cutting each of the compost windrows in three places (east, center, and west of windrow) with a skid-steer loader to expose six vertical faces. Three of the faces (one each from east, center, and west) were sampled at three vertical locations (top, middle, bottom). The vertical location samples from each face were composited to give three subsamples (east, west, center) per windrow. Each sampling time had a total of 12 samples (two bedding types × two replicates × three horizontal locations). In 1999, the sampling protocol varied in that the vertical locations were kept separate. Two faces exposed in the center of the windrow were sampled at three vertical locations (top, middle, bottom). The vertical location samples from each face were then composited to give three samples (top, middle, bottom). Each sampling time had a total of 12 samples (two bedding types × two replicates × three vertical locations).

Bacterial Enumeration

Total coliforms, *E. coli*, and total aerobic heterotrophs (TAH) were enumerated in our study. Total coliform bacteria are excreted in high numbers in animal and human feces, and are often used as indicators of fecal contamination in water and food, even though not all TC bacteria are of fecal origin. We did not use TC data as an indicator of fecal contamination, since clearly, fecal contamination is inapplicable when working with cattle manure. The TC data were used to examine persistence and potential regrowth during the composting process, since the behavior of pathogenic groups may be better represented by TC than by more specific *E. coli* data. We feel that following TC populations in a known experimental sample (manure) as it changes over time (to compost) gives relevant data on environmental fate. *Escherichia coli* is a member of the fecal coliform group, a subset of the TC group, and its presence can be used as a surrogate for specific pathogenic *E. coli* strains. Ogden et al. (2001) reported that since the die-off rate of *E. coli* O157 was the same as that of the commensal *E. coli* population in a laboratory study, the field behavior of *E. coli* O157 in a cattle slurry application study could be approximated by monitoring the population of *E. coli*. Total

aerobic heterotroph levels indicate the overall magnitude of indigenous microbial activity in decomposing organic matter.

Fresh compost samples (10 g wet wt.) were added to 90 mL of sodium phosphate buffer (pH 6.5, 0.05 *M*) and mixed in a Stomacher blender (Model 400; Seward Medical, Mississauga, ON, Canada) for 2 min. The suspension was serially diluted to the appropriate levels in sodium phosphate buffer. The dilutions were inoculated (1 mL) into triplicate Fluorocult LMX broth tubes (9 mL; Merck KGaA, Darmstadt, Germany) and incubated aerobically at 37°C for enumeration of TC and *E. coli* by the most probable number (MPN) method. Total coliforms were enumerated after 48 h as those tubes with hydrolysis of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). *Escherichia coli* was enumerated after 24 and 48 h as those tubes with hydrolysis of both X-Gal and 4-methylumbelliferyl-β-D-glucuronide (MUG). Selected colonies of presumptive TC and *E. coli* were isolated from positive tubes on Fluorocult LMX agar plates for identity confirmation using membrane fatty acid composition, cellular morphology, and biochemical characteristics (Smibert and Krieg, 1994; Paisley 1996; Garthwright, 1998). Samples positive for coliforms were confirmed positive by isolation in 96.7% of the cases. In a few cases coliform bacteria were not isolated, while enterococci were. The data were not corrected for these false positive samples since the TC results would change only marginally. All samples positive for *E. coli* were confirmed positive by pure culture isolation and identification.

The TC and *E. coli* enumerations are presented as $\log_{10}$ cells g^{-1} dry wt. Water content of compost subsamples (oven-drying at 60°C for 48 h) was used to express values on a dry weight basis. For both enumerations, the minimum detection level (MDL) was $\log_{10}$ 0.56 cells g^{-1} wet wt. We assumed an average compost water content of 0.5 kg kg^{-1} to maintain a constant MDL across all sampling dates and present data on a dry weight basis. The MDL of $\log_{10}$ 0.56 cells g^{-1} wet wt. is equivalent to $\log_{10}$ 0.86 cells g^{-1} dry wt. at a compost water content of 0.5 kg kg^{-1}. For determining treatment means, values < MDL were assigned a value of 50% of the MDL (after converting to dry weight), which explains the presence of data points < MDL in the figures presented.

The serial dilutions were also spread-plated (100 μL) in triplicate onto tryptic soy agar plates for enumeration of TAH (as $\log_{10}$ CFU g^{-1} dry wt.) after incubation at 39°C for 48 h.

Compost Temperature and Water Content

Thermocouples and a datalogger (Sciemetric, Nepean, ON, Canada) were installed as soon as the windrows were formed. They were removed just before each turning and reinstalled immediately after turning. Temperatures were logged every 20 min and averaged to give mean daily values. In 1998, three thermocouples (bottom, middle, top) were attached to each of three stainless steel pipes. The pipes were then placed vertically in one replicate of each bedding treatment near the center of the windrow, and about 2 m from the east and west ends. The bottom location was approximately 0.3 m, the center location approximately 0.6 m, and the top location approximately 0.9 m above ground level. Datalogger malfunction resulted in missing temperature data for Days 13 and 14, 30 through 33, and 50. In 1999, temperatures were measured at three vertical locations (as in 1998: bottom, middle, and top) in three windrow positions: the east and west ends of the one replicate of each manure bedding treatment (approximately 25% and 75% along its length), and the center of the other replicate of each treatment. These nine temperatures were averaged to give the bedding treatment means. The bedding × vertical location ($n = 3$) means were also calculated to examine the location effect.

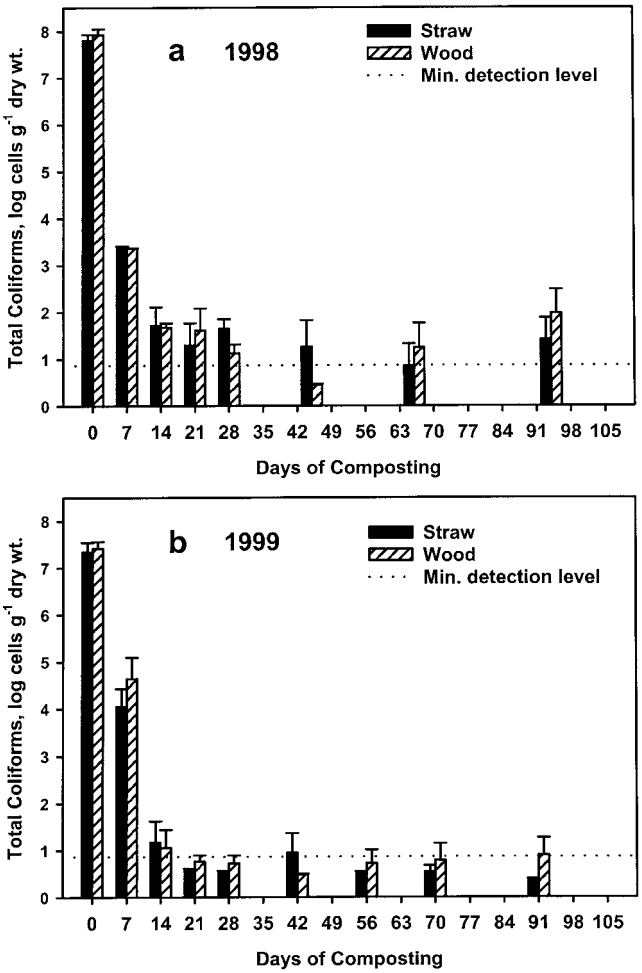

Fig. 1. Effect of bedding type on total coliforms (mean across locations ± SE, $n = 6$) in (*a*) 1998 and (*b*) 1999. Bedding type (1998, 1999) and bedding × location interaction (1999) effects were nonsignificant on all sampling dates.

Although water content was determined on the bacterial compost samples (to convert enumeration data to a dry weight basis), larger compost samples (approximately 0.6 kg) were taken to track water content (after oven-drying at 60°C for 48 h) during composting. In 1998, the six vertical faces were sampled at three (top, middle, and bottom) locations, giving 18 samples per replicate. In 1999, samples were taken at the top, middle, and bottom locations of the two exposed vertical faces giving six samples per replicate.

Statistical Analyses

In 1998 and 1999, the effect of bedding on TC, *E. coli*, and TAH populations was examined using the general linear models procedure (SAS Institute, 1990). Additionally, in 1999, the vertical location effect (bottom, middle, top) was examined as a subtreatment in a split-plot design with bedding as the main treatment.

RESULTS

Total Coliforms

In 1998, there was a large decline in TC as composting progressed and populations were not affected by bedding type on any sampling date (Fig. 1a). Values averaged $\log_{10}$ 7.86 cells g^{-1} (dry wt.) on Day 0, decreasing

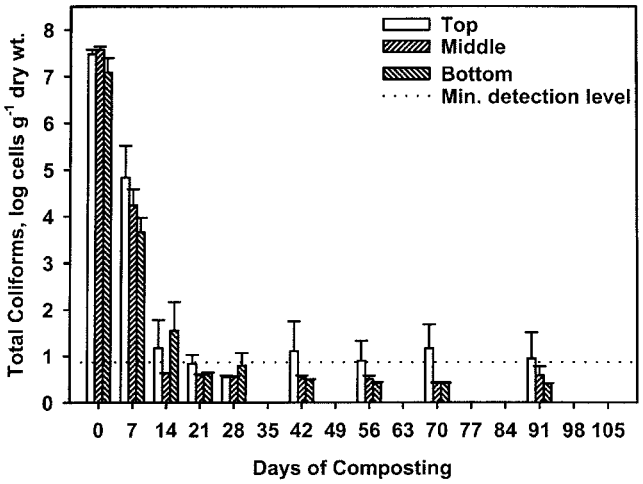

Fig. 2. Effect of vertical windrow location on total coliforms (mean across bedding types ± SE, $n = 4$) in 1999. Bedding type and bedding × location interaction effects were nonsignificant on all sampling dates.

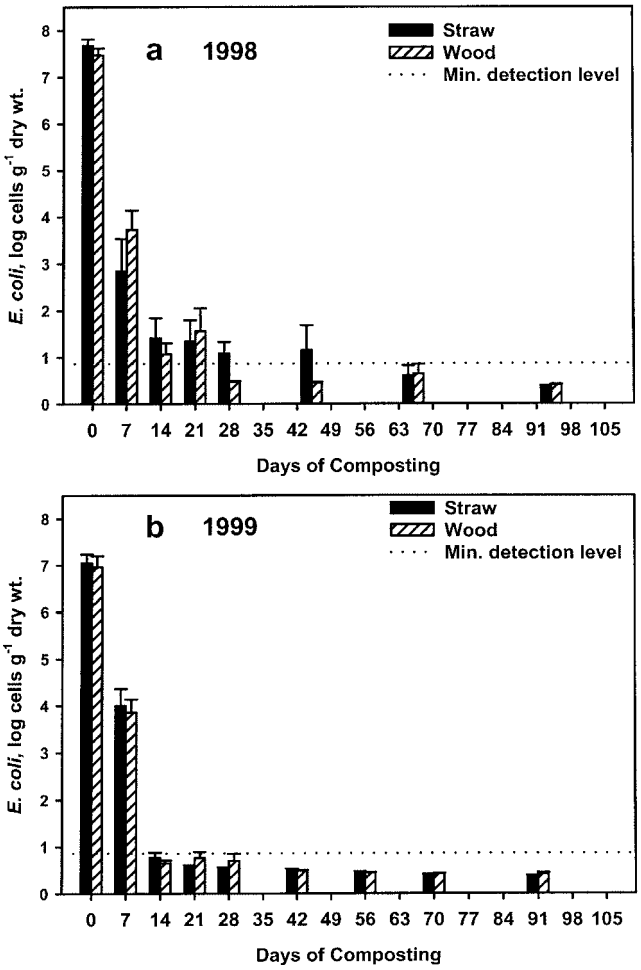

Fig. 3. Effect of bedding type on *E. coli* (mean across locations ± SE, $n = 6$) in (*a*) 1998 and (*b*) 1999. Bedding type (1998, 1999) and bedding × location interaction (1999) effects were nonsignificant on all sampling dates.

to $\log_{10}$ 3.38 cells g^{-1} by Day 7, and $\log_{10}$ 1.69 cells g^{-1} by Day 14. To put this in perspective, 99.997% of TC were eliminated in the first 7 d. The TC were detectable on all sampling dates except for the wood chip–bedded compost on Day 45. On Day 94, TC levels averaged exactly the same as Day 14, and about sevenfold higher than Day 45 ($\log_{10}$ 0.86 cells g^{-1}), indicating some regrowth. In 1999, the effect of bedding type on TC was also nonsignificant (Fig. 1b). The trend with time was similar to 1998 in that TC levels declined significantly in the early stages of composting (approximately 3 $\log_{10}$ units from Days 0–7, and a further 3.2 $\log_{10}$ units from Days 7–14). The slight resurgence in TC levels, evident toward the end of active composting in 1998, did not occur in 1999 (Fig. 1b), possibly because the population declined to a greater extent. The TC levels were <MDL in straw- and wood chip–bedded material by Day 21 in 1999 (Fig. 1b), but were detectable until Day 94 in 1998 (Fig. 1a).

Composting guidelines (Canadian Council of Ministers of the Environment, 1996) dictate that finished compost should contain <$\log_{10}$ 3.0 cells g^{-1} dry wt. of fecal coliforms, which is a subset of TC. Our final average TC values of $\log_{10}$ 1.69 cells g^{-1} in 1998 and $\log_{10}$ 0.64 cells g^{-1} in 1999 were well below the guidelines. In 1999, the top location had a trend of higher levels of TC than the middle and bottom locations (e.g., Day 7), but the effect was nonsignificant (Fig. 2) on all sampling dates.

Escherichia coli

In 1998, *E. coli* levels were not affected by bedding type (Fig. 3a). The decline of *E. coli* with composting followed a similar trend to that for TC. *Escherichia coli* levels averaged $\log_{10}$ 7.57 cells g^{-1} (dry wt.) on Day 0, $\log_{10}$ 3.29 cells g^{-1} on Day 7, and $\log_{10}$ 1.24 cells g^{-1} on Day 14. This meant that 99.995% of *E. coli* was eliminated in the first 7 d. *Escherichia coli* was still detectable on Day 45 for straw-bedded compost but was nondetectable after Day 21 on wood chip–bedded compost (Fig. 3a). In 1999, the *E. coli* trends were similar with a nonsignificant bedding effect (Fig. 3b) and a dramatic drop in levels in the early stages of composting. Values fell from an average $\log_{10}$ 7.01 cells g^{-1} on Day 0, to $\log_{10}$ 3.93 cells g^{-1} on Day 7, and $\log_{10}$ 0.71 cells g^{-1} on Day 14. Additionally, *E. coli* was <MDL on both bedding types by Day 14, which was earlier than in 1998. The final *E. coli* values at the end of active composting were <MDL and identical in both years ($\log_{10}$ 0.40 cells g^{-1}). Unlike TC, there was no resurgence of *E. coli* toward the end of active composting in either year. The vertical location effect on *E. coli* populations in 1999 was nonsignificant on all sampling dates except Day 14 (Fig. 4), when the bottom location was significantly higher ($\log_{10}$ 0.94 cells g^{-1}) than the middle and top locations ($\log_{10}$ 0.57–0.62 cells g^{-1}). Since all these levels are low, the practical significance of this finding may be marginal.

Total Aerobic Heterotrophs

Initial total aerobic heterotroph (TAH) values (average of two bedding types, Day 0) in 1998 were $\log_{10}$ 9.59 CFU g^{-1} (dry wt.), dropping to a low of $\log_{10}$ 7.61 CFU

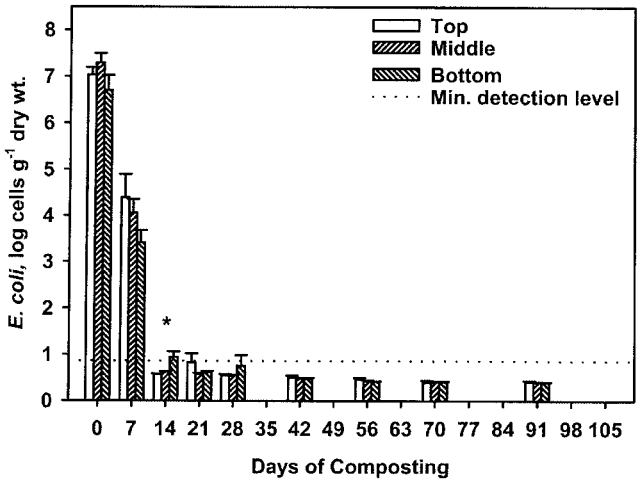

Fig. 4. Effect of vertical windrow location on *E. coli* (mean across bedding types ± SE, $n = 4$) in 1999. The symbol * indicates significance at the 0.05 probability level. Bedding × location interaction effect was nonsignificant on all sampling dates.

g^{-1} on Day 14, and increasing to $\log_{10}$ 8.18 CFU g^{-1} on Day 94 (Fig. 5a). In 1999, a similar trend occurred, with average values being highest on Day 0 ($\log_{10}$ 8.97 CFU g^{-1}), lower by approximately 2 $\log_{10}$ units on Day 14, and then increasing again on Day 91 (Fig. 5b). Of the bacterial groups studied (TC, *E. coli*, and TAH), TAH was the only one with a significant bedding effect on populations. In 1998, the straw-bedded material had significantly ($P = 0.05$) higher TAH than wood chip–bedded material on Day 21 (Fig. 5a). However, on Day 94, the opposite was true. In 1999, the wood chip–bedded material had significantly higher TAH than straw-bedded material on Days 0, 21, 28, and 42 (Fig. 5b).

Windrow Temperature and Water Content

The straw-bedded treatment temperature peaked at 68.7°C on Day 23 in 1998 (Fig. 6a), and 60.6°C on Day 47 in 1999 (Fig. 6b). The wood chip–bedded treatment peaked at 66.7°C on Day 45 in 1998 (Fig. 6a) and 59.8°C on Day 44 in 1999 (Fig. 6b). Daily mean temperature (DMT) for both bedding treatments was generally warmer in 1998 than in 1999. For straw-bedded compost, 45 of 108 d had a DMT of >55°C in 1998. In contrast, there were only 24 of 99 d with a DMT of >55°C in 1999. For wood chip–bedded compost, 44 d had a DMT of >55°C in 1998 compared with 30 d in 1999. These values also show that there was little difference in temperature regime due to bedding type. If a DMT of <40°C is used as an indicator of the end of thermophilic composting, then this occurred on Day 108 for straw- and Day 91 for wood chip–bedded compost in 1998 and on Day 83 for straw- and Day 87 for wood chip–bedded compost in 1999.

Water was not added to the windrows during the active composting phase. In 1998, water content of the straw-bedded manure was not significantly different (0.67 kg kg^{-1} wet wt.) than that of the wood chip–bedded manure (0.61 kg kg^{-1}) on Day 0 or on any of the remaining bacteria sampling dates (Table 1). By Day 21, both manures had dried to water contents of 0.49 kg kg^{-1} and they continued to lose water until the end of active composting. In 1999, water content was significantly higher on Day 0 for straw-bedded (0.65 kg kg^{-1}) than wood chip–bedded manure (0.60 kg kg^{-1}) but differences were nonsignificant thereafter (Table 1). Water content was generally higher at all points in the composting process than in 1998.

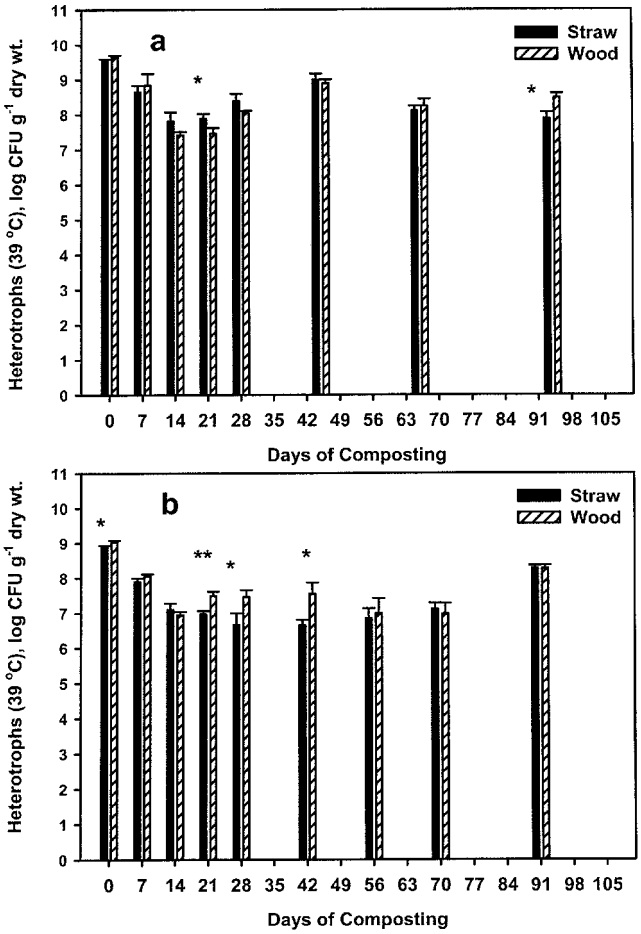

Fig. 5. Effect of bedding type on total aerobic heterotroph populations incubated at 39°C (mean ± SE, $n = 6$) in (*a*) 1998 and (*b*) 1999. The symbols * and ** indicate bedding effect significance at the 0.05 and 0.01 probability levels, respectively.

DISCUSSION

Populations of TC and *E. coli* were not affected by bedding type on any sampling date in either year, even though wood products contain antimicrobial compounds such as phenols and tars (Allison and Anderson, 1951). Our findings agree with those of Miller et al. (2003), working at the same feedlot. They found no difference in TC levels in raw manure samples from feedlot pen floors bedded with straw or wood chips. In contrast, Kudva et al. (1998) indicated that small amounts of wood chip bedding (<5%) in bovine manure may have contributed to shorter survival times for *E. coli* O157:H7. Wood chip bedding represented an average

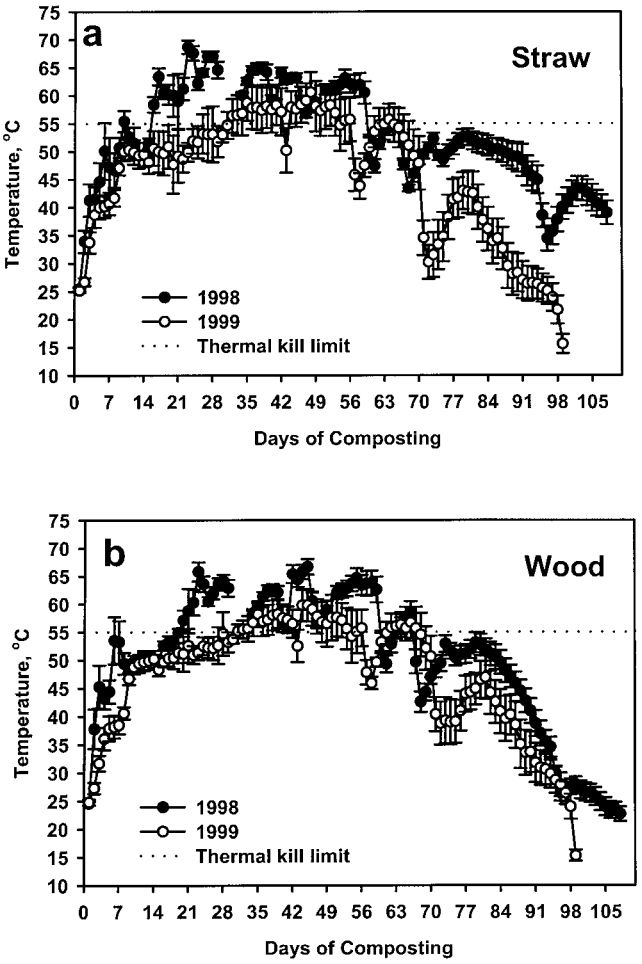

Fig. 6. Effect of year on windrow temperature (mean ± SE, n = 9) during composting of (*a*) straw-bedded and (*b*) wood chip–bedded manure.

of 22%, and straw an average of 18% of total manure dry weight in our study (unpublished data, 1999). However, this level of wood chip bedding did not lower levels of TC compared with straw bedding.

The regrowth of TC between Day 45 and Day 94 in 1998 agrees with the findings of Hassen et al. (2001), who reported a phase of resurgent growth of fecal coliforms after 9 wk of MSW composting. They attributed this secondary growth to recontamination or redistribution during windrow turning. Krogmann et al. (2002) found that after an initial population decrease in fecal streptococci (*Enterococcus* spp.), a slight increase (approximately 10-fold) was observed in later composting stages of horse (*Equus caballus*) manure. Their explanation of this included (i) normal data variability, (ii) pile contamination by turning with dirty equipment or by vermin between sampling dates, or (iii) recontamination of sanitized compost by unsanitized material from the outer mesophilic area of the pile during turning. Any, or all, of these reasons may explain regrowth in our study.

The time–temperature profiles in our study were typical for composting of organic materials and similar to those reported for open-windrow composting of feedlot manure in Nebraska (Eghball et al., 1997). There was

Table 1. Effect of bedding type (straw, wood chips) on windrow water content during composting in 1998 (n = 36) and 1999 (n = 12).

	Water content	
Day	Straw	Wood Chips
	kg kg^{-1}	
	1998	
0	0.665a†	0.612a
7	0.601a	0.572a
14	0.569a	0.544a
21	0.488a	0.492a
28	0.404a	0.427a
45	0.332a	0.374a
66	0.276a	0.328a
94	0.259a	0.314a
	1999	
0	0.651a	0.595b
7	0.638a	0.596a
14	0.591a	0.571a
21	0.570a	0.545a
29	0.504a	0.512a
42	0.382a	0.425a
56	0.359a	0.393a
70	0.292a	0.354a
98	0.287a	0.318a

† Within years and days, means followed by the same letter are not significantly different from each other (P = 0.05).

an early rapid rise to thermophilic conditions, slight temperature decreases followed by partial recovery associated with each turning event, and then a gradual cooling of the windrows toward the end of active composting. However, there was little benefit of prolonged exposure above the recognized thermal kill limit of 55°C, as most TC and *E. coli* were eliminated in the first 7 d of composting before attaining 55°C. Additionally, the higher residual levels of TC (Fig. 1) observed toward the end of composting in 1998 compared with 1999 were not due to cooler conditions, as there were warmer temperatures in 1998 compared with 1999 (Fig. 5).

Since most of the coliform population decline occurred in the first 7 d of composting, temperature conditions during this period were examined more closely (Table 2). In 1998, the average temperature was 39.5°C in the straw-bedded and 41.5°C in the wood chip–bedded compost during the first 7 d. Maximum tempera-

Table 2. Average temperature and maximum temperature as affected by bedding type (1998 and 1999) and vertical windrow location (1999) in the initial 7 d of composting.

Bedding	Average temperature	Maximum temperature
	°C	
	Bedding effect in 1998	
Straw	39.5 (±3.2)†	50.1
Wood chips	41.5 (±3.7)	53.5
	Bedding effect in 1999‡	
Straw	35.0 (±2.5)	40.7
Wood chips	33.5 (±2.1)	38.5
	Vertical location effect in 1999§	
Top	36.5 (±2.4)	42.2
Middle	36.0 (±2.9)	43.6
Bottom	30.2 (±1.8)	35.8

† Standard errors of the means are in parentheses (n = 7).
‡ Averaged across locations.
§ Averaged across bedding types.

tures were 50.1°C for straw-bedded and 53.5°C for wood chip–bedded compost. In 1999, average temperatures for the first 7 d were 35°C for straw-bedded and 33.5°C for wood chip–bedded and the maxima were 40.7°C for straw-bedded and 38.5°C for wood chip–bedded compost (Table 2). The coolest temperature regime in either year was at the bottom windrow location in 1999, which had an average temperature of 30.2°C and a maximum temperature of 35.8°C during the first 7 d. This regime led to a decrease in TC from $\log_{10}$ 7.08 to $\log_{10}$ 3.66 cells g^{-1} (dry wt.) and a decrease in *E. coli* from $\log_{10}$ 6.7 to $\log_{10}$ 3.41 cells g^{-1}. Hassen et al. (2001) reported a decrease from $\log_{10}$ 7.40 cells g^{-1} (dry wt.) to $\log_{10}$ 3.90 cells g^{-1} in municipal solid waste compost at 55 to 60°C over 15 wk. Our *E. coli* levels declined a similar magnitude at much lower temperatures in just 7 d. Our findings were closer to those of Lung et al. (2001), who found that *E. coli* O157:H7 at levels of $\log_{10}$ 7.0 CFU g^{-1} in raw cow manure was not detected after 72 h of composting at 45°C.

The optimum water content for windrow manure composting is 0.4 to 0.65 kg kg^{-1} (Rynk, 1992), since dehydration results in inactivation of beneficial as well as pathogenic microbes. Himathongkham and Riemann (1999) reported that the destruction of *E. coli* was greatly increased by the drying of chicken manure to a water content of 0.10 kg kg^{-1}. Although overall water losses during summer windrow composting of feedlot manure in southern Alberta can be substantial (approximately 75%), due to turning combined with high evaporation rates (Larney et al., 2000), the water contents of our composts showed little change in the first 7 d, when most of the decline in coliform levels occurred. In 1998, water content fell from 0.67 kg kg^{-1} on Day 0 to 0.60 kg kg^{-1} on Day 7 for straw-bedded compost and from 0.61 to 0.57 kg kg^{-1} for wood chip–bedded compost. In 1999, water contents were generally stable in the first 7 d declining only slightly (0.65 to 0.64 kg kg^{-1}) on the straw-bedded compost (Table 1).

Lack of nutrients caused by high populations of indigenous microorganisms in manure or the production of compounds detrimental to coliforms may also play a role in the decline of pathogens during composting (Himathongkham et al., 1999). Pietronave et al. (2002) demonstrated that indigenous microflora suppressed seeded *E. coli* growth in a nonsterilized finished compost while *E. coli* grew rapidly in sterilized compost. Sidhu et al. (2001) reported an antagonistic effect of indigenous microorganisms on *Salmonella* in composted biosolids. However, the antagonistic effect declined with duration of storage and hence long-term storage was not recommended. In our study, the maintenance of microbial activity and competition for nutrients by TAH throughout the composting period may have played a role in suppressing TC and *E. coli*. However, since the significant bedding effects on TAH populations did not translate into significant effects on TC or *E. coli* levels, the role of this inactivation mechanism may have been small.

Another mechanism of pathogen reduction is pH change. Ugwuanyi et al. (1999) found that *E. coli* was more sensitive to elimination at pH 7 than pH 8 during aerobic digestion at 55°C. Our straw-bedded manure had a pH 8.1, while wood chip–bedded manure had a pH 7.3 on Day 0 in 1998 (Larney et al., 2001). The pH values decreased to 7.3 on Day 14 for the straw-bedded manure and pH 6.8 on Day 14 for the wood chip–bedded manure, which may have enhanced the pathogen reduction effect.

CONCLUSIONS

During open-air windrow composting of beef feedlot manure in southern Alberta, we achieved 10^2– to 10^4–fold reductions in bacterial levels (TC and *E. coli*) in 7 d and 10^5– to 10^7–fold reductions in 14 d. Even though our straw- and wood chip–bedded composts remained thermophilic for >80 d in both study years, this prolonged exposure was not required for pathogen elimination. More than 99.9% of TC and *E. coli* was eliminated in the first 7 d when average windrow temperatures were 33.5 to 41.5°C, which is within the mesophilic range and 14 to 22° lower than the thermal kill limit of 55°C in composting guidelines (USEPA, 1992; Canadian Council of Ministers of the Environment, 1996). These guidelines also specify maintenance of 55°C for at least 15 d. While this target was easily exceeded in our study (24–45 d > 55°C), it was not essential to achieve the observed levels of pathogen reduction.

Total coliforms and *E. coli* were not significantly affected by bedding type (straw vs. wood chips) at any time. Total coliforms were detectable until Day 94 in 1998 ($\log_{10}$ 1.69 cells g^{-1} dry wt.). Although this level is well below established guidelines (<$\log_{10}$ 3.0 cells g^{-1} dry wt. of fecal coliforms [Canadian Council of Ministers of the Environment, 1996]), it shows that there may be some potential for regrowth of coliforms at the later stages of composting. *Escherichia coli* was <MDL after Day 45 in 1998 and after Day 7 in 1999. Dessication probably played a minor role in coliform elimination, since water loss was low (<0.07 kg kg^{-1}) in the first 7 d of composting when most of the elimination occurred. Total aerobic heterotroph populations remained high (>7.0 $\log_{10}$ CFU g^{-1} dry wt.) throughout the composting period and their competition for nutrients may have caused an antagonistic effect on pathogens.

In reducing coliform populations by >99.9%, land application of compost instead of raw manure should significantly reduce the risk of water quality degradation in areas of intensive livestock production, like southern Alberta. Although pathogen reduction is a recognized benefit of composting, our quantification of its magnitude, as well as the duration and temperature regimes required for typical feedlot manures under southern Alberta conditions, adds to our knowledge base on composting as a manure management alternative in the region.

ACKNOWLEDGMENTS

This study was supported in part by the Canada-Alberta Beef Industry Development Fund. We thank Andrew Olson and Paul DeMaere for compost windrow management, Bruce

Beasley and Brian Handerek for compost sampling, and Troy Bech for bacterial enumeration.

REFERENCES

Allison, F.E., and M.S. Anderson. 1951. The use of sawdust for mulches and soil improvement. Circ. 891. USDA, Washington, DC.

Bach, S.J., T.A. McAllister, D.M. Veira, V.P.J. Gannon, and R.A. Holley. 2002. Transmission and control of *Escherichia coli* O157: H7— A review. Can. J. Anim. Sci. 82:479–490.

Canadian Council of Ministers of the Environment. 1996. Support document for compost quality criteria. National Standard of Canada (CAN/BNQ 0413-200). The Canadian Council of Ministers of the Environment (CCME) guidelines and Agriculture and Agri-Food Canada (AAFC) criteria. Available online at http://www.compost.org/compostqualitydoc.pdf (verified 17 Feb. 2003). CCME, Winnipeg, MB.

Déportes, I., J.L. Benoit-Guyod, D. Zmirou, and M.C. Bouvier. 1998. Microbial disinfection capacity of municipal solid waste (MSW) composting. J. Appl. Microbiol. 85:238–246.

Droffner, M.L., and W.F. Brinton. 1995. Survival of *E. coli* and *Salmonella* populations in aerobic thermophilic composts as measured with DNA gene probes. Zentralbl. Hyg. Umweltmed. 197:387–397.

Droffner, M.L., W.F. Brinton, and E. Evans. 1995. Evidence for the prominence of well characterized mesophilic bacteria in thermophilic (50–70 °C) composting environments. Biomass Bioenergy 8:191–195.

Eghball, B., J.F. Power, J.E. Gilley, and J.W. Doran. 1997. Nutrient, carbon, and mass loss during composting of beef cattle feedlot manure. J. Environ. Qual. 26:189–193.

Entry, J.A., and N. Farmer. 2001. Movement of coliform bacteria and nutrients in ground water flowing through basalt and sand aquifers. J. Environ. Qual. 30:1533–1539.

Epstein, E. 1997. The science of composting. Technomic Publ. Co., Lancaster, PA.

Gagliardi, J.V., and J.S. Karns. 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. Appl. Environ. Microbiol. 66:877–883.

Garthwright, W.E. 1998. Food and Drug Administration bacteriological analytical manual. 8th ed. Revision A. Appendix 2. AOAC Int., Gaithersburg, MD.

Hassen, A., K. Belguith, N. Jedidi, A. Cherif, M. Cherif, and A. Boudabous. 2001. Microbial characterization during composting of municipal solid waste. Bioresour. Technol. 80:217–225.

Himathongkham, S., S. Bahari, H. Riemann, and D. Cliver. 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiol. Lett. 178:251–257.

Himathongkham, S., and H. Riemann. 1999. Destruction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia. FEMS Microbiol. Lett. 171:179–182.

Krogmann, U., P.V. Romano, and M.L. Westendorf. 2002. Reduction of fecal streptococci and strongyle eggs during small-scale composting of horse manure mixed with wood shavings. *In* F.C. Michel et al. (ed.) Proc. Int. Symp. Composting and Compost Utilization, Columbus, OH. 6–8 May 2002. [CD-ROM computer file.] The JG Press, Emmaus, PA.

Kudva, I.T., K. Blanch, and C.J. Hovde. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol. 64:3166–3174.

Larney, F.J., and R.E. Blackshaw. 2003. Weed seed viability in composted beef cattle feedlot manure. J. Environ. Qual. 32:1105–1113.

Larney, F.J., C. Chang, H.H. Janzen, J.J. Miller, S.M. McGinn, B.S. Freeze, and J.J. Leonard. 2001. Managing feedlot manure using composting technology. Final report. Project 97AB063. Canada-Alberta Beef Industry Development Fund, Alberta Cattle Commission, Calgary.

Larney, F.J., A.F. Olson, A.A. Carcamo, and C. Chang. 2000. Physical changes during active and passive composting of beef feedlot manure in winter and summer. Bioresour. Technol. 75:139–148.

Lung, A.J., C.M. Lin, J.M. Kim, M.R. Marshall, R. Nordstedt, N.P. Thompson, and C.I. Wei. 2001. Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. J. Food Prot. 64:1309–1314.

McAllister, T.A., F.J. Larney, J.J. Miller, L.J. Yanke, and I. Walker. 1998. Wood chips vs. straw for bedding. Canadian Cattlemen—The Beef Magazine 61(10A):26–30.

Miller, J.J., B. Beasley, L.J. Yanke, F.J. Larney, T.A. McAllister, B.M. Olson, L.B. Selinger, D.S. Chanasyk, and P. Hasselback. 2003. Bedding and seasonal effects on chemical and bacterial quality of feedlot cattle manure. J. Environ. Qual. (in press).

Mote, C.R., B.L. Emerton, J.S. Allison, H.H. Dowlen, and S.P. Oliver. 1988. Survival of coliform bacteria in static compost piles of dairy waste solids intended for freestall bedding. J. Dairy Sci. 71:1676–1681.

O'Connor, D.R. 2002. Part 1. Report of the Walkerton Inquiry: The events of May 2000 and related issues. Ontario Ministry of the Attorney General, Queen's Printer of Ontario, Toronto.

Ogden, I.D., D.R. Fenlon, A.J.A. Vinten, and D. Lewis. 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. Int. J. Food Microbiol. 66:111–117.

Paisley, R. 1996. MIS whole cell fatty acid analysis by gas chromatography: Training manual. MIDI, Newark, DE.

Pell, A.N. 1997. Manure and microbes: Public and animal health problem? J. Dairy Sci. 80:2673–2681.

Pietronave, S., L. Fracchia, and M.G. Martinotti. 2002. The role of indigenous microflora in the suppression of human pathogen regrowth in a finished compost. *In* F.C. Michel et al. (ed.) Proc. Int. Symp. Composting and Compost Utilization, Columbus, OH. 6–8 May 2002. [CD-ROM computer file.] The JG Press, Emmaus, PA.

Rynk, R. 1992. On-farm composting handbook. Publ. NRAES-54. Northeast Regional Agric. Eng. Serv., Ithaca, NY.

SAS Institute. 1990. SAS user's guide. Statistics. Version 6 ed. SAS Inst., Cary, NC.

Schleiff, G., and W. Dorn. 1997. Hygienic–bacteriologic evaluation of methods for production of dry poultry feces manure. Zentralbl. Hyg. Umweltmed. 199:475–495.

Sidhu, J., R.A. Gibbs, G.E. Ho, and I. Unkovich. 2001. The role of indigenous microorganisms in suppression of *Salmonella* regrowth in composted biosolids. Water Res. 35:913–920.

Smibert, R.M., and N.R. Krieg. 1994. Phenotypic characterization. p 607–654. *In* P. Gerhardt et al. (ed.) Methods for general and molecular bacteriology. Am. Soc. Microbiol., Washington, DC.

Tiquia, S.M., J.H.C. Wan, and N.F.Y. Tam. 2002. Microbial population dynamics and enzyme activities during composting. Compost Sci. Util. 10:150–161.

Turner, C. 2002. The thermal inactivation of *E. coli* in straw and pig manure. Bioresour. Technol. 84:57–61.

Ugwuanyi, J.O., L.M. Harvey, and B. McNeil. 1999. Effect of process temperature, pH and suspended solids content upon pasteurization of a model agricultural waste during thermophilic aerobic digestion. J. Appl. Microbiol. 87:387–395.

USEPA. 1992. Standards for the use or disposal of sewage sludge. Regulation 503. Final. USEPA, Washington, DC.