Determining Thermal Inactivation of *Escherichia coli* O157:H7 in Fresh Compost by Simulating Early Phases of the Composting Process

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A three-strain mixture of *Escherichia coli* O157:H7 was inoculated into fresh dairy compost (ca. 10^7 CFU/g) with 40 or 50% moisture and was placed in an environmental chamber (ca. 70% humidity) that was programmed to ramp from room temperature to selected composting temperatures in 2 and 5 days to simulate the early composting phase. The surviving *E. coli* O157:H7 population was analyzed by direct plating and enrichment. Optimal and suboptimal compost mixes, with carbon/nitrogen (C/N) ratios of 25:1 and 16:1, respectively, were compared in this study. In the optimal compost mix, *E. coli* O157:H7 survived for 72, 48, and 24 h in compost with 40% moisture and for 72, 24, and 24 h with 50% moisture at 50, 55, and 60°C, respectively, following 2 days of come-up time (rate of heating up). However, in the suboptimal compost mix, the pathogen survived for 288, 72, and 48 h in compost with 40% moisture and for 240, 72, 24 h in compost with 50% moisture at the same temperatures, respectively. Pathogen survival was longer, with 5 days of come-up time compared with 2 days of come-up. Overall, *E. coli* O157:H7 was inactivated faster in the compost with 50% moisture than in the compost with 40% at 55 and 60°C. Both moisture and come-up time were significant factors affecting Weibull model parameters. Our results suggest that slow come-up time at the beginning of composting can extend pathogen survival during composting. Additionally, both the C/N ratio and the initial moisture level in the compost mix affect the rate of pathogen inactivation as well.

Livestock wastes, e.g., manures that have undergone appropriate treatment to inactivate human pathogens, can be a safe soil amendment for use in agriculture. However, inadequate treatment of such manure may lead to the survival of pathogens that could contaminate produce in the field and ultimately result in food-borne illness. *Escherichia coli* O157:H7 is one of the most important and common pathogens, responsible for many of the food-borne illnesses in the United States (18). Animals carry this pathogen without apparent symptoms and may also sporadically shed the bacteria (12), which can be disseminated to the environment. In the past, several outbreaks of food-borne illnesses have been linked to the contamination of produce, such as garden vegetables and baby spinach, via direct or indirect contact with animal wastes containing human pathogens (2, 4, 5).

Composting is commonly used for treating organic wastes (livestock manure, food wastes, etc.), which makes them easier to dispose of on agricultural fields and home gardens. Composted organic waste serves as an important organic fertilizer, which is rich in nutrients, circumventing the need for chemical fertilizers. Heat generated from the metabolic activity of the microbes present in a compost mixture plays a major role in the inactivation of zoonotic pathogens. Therefore, composting is considered important in bringing about inactivation/killing of pathogens that may be present in livestock wastes. However, the primary process criteria used for ensuring the microbiological safety of composts have been narrowly defined as temperature conditions. In the United States, EPA regulations for composting of biosolids include either a minimum temperature of 55°C for 3 days in aerated static piles or in-vessel systems or for 15 days with 5 turnings in windrow systems (24).

Although temperature is a critical factor during composting, extended survival of pathogens in compost has been reported. Droffner and Brinton (8) reported that in bench-scale trials, *E. coli* B survived for at least 9 days at 60 to 70°C in a biowaste (food waste) compost or a wastewater sludge compost and *Salmonella enterica* serovar Typhimurium Q survived for at least 9 and 5 days over 60°C in the food biowaste compost and the wastewater sludge compost, respectively. Hutchison et al. (14) has also reported the extended survival of pathogens in field studies of static compost piles. In that study, *Salmonella, E. coli* O157:H7, and *Listeria* survived for more than 8 days in poultry manure-based compost piles when exposed to temperatures above 55°C. These studies suggest that the time-temperature criteria set by the EPA may not always be sufficient to ensure complete inactivation of pathogens within the entire compost pile.

Pathogen inactivation during composting is very complex. Besides elevated temperature, composting is affected by other factors, such as moisture content, carbon/nitrogen ratio (C/N), particle size, aeration, heap size, pH, and types and populations of indigenous microflora. The optimal moisture and C/N ratios for active composting are 50 to 60% and 25:1 to 30:1, respectively, however, 40 to 65% and 20:1 to 40:1, respectively,
are acceptable (22). Variations of these factors may affect the rapid onset of self-heating at the beginning of composting, causing slow heat-up and extend the transition time from the mesophilic to the thermophilic phase of composting. Consequently, some populations of pathogenic bacteria may become acclimated before lethal temperatures are reached, or even survive, for an extended period of time (1, 7). Therefore, relying solely on time-temperature criteria for pathogen inactivation without taking into consideration other composting factors may not completely ensure compost safety.

The objectives of this study were to investigate the effect of some composting parameters, i.e., initial moisture level, C/N ratio, and rate of heating up (come-up time) on the thermal inactivation of E. coli O157:H7 in fresh dairy compost under a controlled environment, and develop predictive models to analyze the thermal inactivation data.

MATERIALS AND METHODS

Compost preparation. Fresh compost mixtures were prepared by mixing dairy manure (collected from LaMaster dairy farm, Clemson University), sawdust bedding, and hay at different ratios to yield compost mixture with a C/N ratio of 25:1 (optimal ratio) or 16:1 (suboptimal ratio). The C/N ratio of the compost was analyzed by the Agricultural Service Laboratory (Clemson University, Clemson, SC). The compost mixture was stored under refrigeration conditions until used.

Two days prior to the experiment, the refrigerated compost mixture was split into two lots and dried under an airflow supreme fume hood (Kewaunee Scientific Equipment corp., Michigan) to reduce the moisture to ca. 50% (for optimal composting conditions) and 40% (for suboptimal composting conditions). The compost moisture was measured by an IR-35 moisture analyzer (Denver Instrument, Germany).

Bacterial culture preparation. E. coli O157:H7 strains F06M-0923-21 (spinach outbreak strain from the California Department of Health), F07M-020-1 (Taco John’s outbreak strain from the California Department of Health), and avirulent outbreak strain from the California Department of Health), F07M-020-1 (Taco John’s outbreak strain from the California Department of Health), and avirulent outbreak strain from the California Department of Health), were induced to rifampin resistance by an IR-35 moisture analyzer (Denver Instrument, Germany).

About 100 g of inoculated compost with 40 or 50% moisture at a final concentration of ca. 10^7 CFU/g separately, with the use of a spray nozzle sanitized with 70% ethanol and rinsed with sterile saline. The compost was mixed continuously for 10 min on sterile polypropylene trays by hand, wearing sterile gloves.

Thermal inactivation study. About 100 g of inoculated compost with 40 or 50% moisture content was put into a 2.2 kg Tyvek pouch (size, 5.25 in. by 10 in.; SPS Medical, Rush, NY) and spread evenly into a thin layer (ca. 1 cm in depth). Tyvek pouches were then kept in a single layer on the shelf of an environmental chamber (model no. EC2047N; Thermo Scientific, Barnstead International, Dubuque, IA), with the humidity set at ca. 70% to mimic conditions inside the composting heap. The temperatures used for this study were 50, 55, and 60°C, which were monitored constantly using type T thermocouples (DCC Corporation, New Jersey) with one cord inserted inside the compost pouch, and others were kept in the chamber. The temperature rise of the environmental chamber during the study was programmed to ramp stepwise from room temperature (ca. 26°C) to the target temperature in 2 days (representing a normal temperature rise during the composting process) or 5 days (slow heat-up). After the temperature of the compost inside the bag reached the target temperature, sample bags were removed at predetermined time intervals and cooled immediately in an ice water bath.

A 25-g portion of compost sample was taken and mixed with 225 ml of universal preenrichment broth (UPB) (Acumedia Manufacturers Inc., Lansing, MI) in a stomacher bag and homogenized. Serial dilutions of sample homogenates were plated in duplicate on TSA-R to analyze the surviving population of E. coli O157:H7. The detection limit for plating was 25 CFU/g. The samples, which were negative for E. coli O157:H7 after direct plating, were preenriched in UPB, followed by selective enrichment in TSB-R at 37°C overnight, and then streaked on TSA-R and Sorbitol MacConkey agar with rifampin (SMAC-R). The detection limit for enrichment was 0.02 CFU/g. The presumptive colonies on the plates were confirmed to be E. coli O157:H7 using an immune latex agglutination test (Oxoid, Hampshire, United Kingdom). Two or three trials were conducted for each experiment.

Statistical analysis. To compare the difference in bacterial populations of different treatments, plate count data were converted to log_{10} values and subjected to analysis of variance with a test criterion (F statistic) and type I error controlled at a P value of 0.05. The Tukey multiple-comparison procedure of the Statistical Analysis System (2001; SAS, Cary, NC) was used.

Data fitting for thermal inactivation of E. coli O157:H7 in fresh compost. The plate count data for the isothermal inactivation study were converted to log_{10} values and were subjected to a mixed Weibull distribution as described previously (23). The inactivation rate of E. coli O157:H7 at different temperatures was reported as the 4D value (time required to reduce 4 logs of pathogen at different temperatures).

Analyzing factors on parameters of the mixed Weibull model. In order to examine the effects of different factors, including temperature, moisture, come-up time, and C/N ratio on each parameter in the mixed Weibull model, two regression studies were performed using the fitting results of different experimental results. First, the stepwise regression tool in the MATLAB software program (The MathWorks Inc., Natick, MA) was used to identify statistically significant factors. For each factor, a t-statistics test was performed on regression coefficients and the P-value threshold of 0.05 was used to determine the significant factors. Then, the trend in MATLAB was used to fit the interaction response surfaces in order to understand the relationship between factors.

RESULTS

Thermal inactivation of E. coli O157:H7 in fresh compost was performed inside an environmental chamber by simulating the early phase of optimal composting (2 days of come-up time) compared to the early phase of suboptimal composting (5 days of come-up time). The effects of the moisture content, i.e., 50% (optimal) and 40% (suboptimal), along with different compost C/N ratios of 25:1 (optimal) and 16:1 (suboptimal), were also compared.

Comparison of young culture and low-nutrient-adapted cultures for thermal resistance. Initially, the thermal inactivation of the young culture (YC) and that of the low-nutrient-adapted culture (LC) (grown in 1:10-strength TSB-R) were compared at different composting temperatures. With 2 days of come-up time, both YC and LC of E. coli O157:H7 in compost with 50 and 40% moisture survived for 72 and 24 h at 50 and 60°C, respectively, and for 24 and 48 h for 50 and 40% moisture, respectively, at 55°C (Tables 1, 2, and 3). The compost mix with 50% moisture content and with 2 days of come-up time had a slightly quicker decline (lower 4D value) in the LC population than that of the YC (higher 4D value) at 60°C (see Table 6).

The same trend was observed for YC and LC at 60°C in compost with 40% moisture content as well. In the compost mix with 50 and 40% moisture, the differences in survival between YC and LC were not significant (P > 0.05) for most of the sampling times at 50 and 55°C with 2 days of come-up time, except at times 0 and 2 h of inactivation at 50°C in compost mix with 40% moisture and 2 days of come-up time (Tables 1 and 2). At 60°C, there was a significant (P < 0.05)
difference in the survival of the YC and that of the LC at most of the sampling times in compost with 40% moisture and only at 0 and 0.5 h in compost with 50% moisture (Table 3). Since the YC survived equally well or slightly better than the LC, the YC was used for the rest of the composting trials.

**E. coli O157 inactivation in fresh compost at a C/N ratio of 25:1.** For 2 days of come-up time, *E. coli* O157:H7 was inoculated into the compost with a C/N ratio of 25:1 and moisture of 50% at levels of ca. 7.16 ± 0.16, 7.23 ± 0.13, and 7.04 ± 0.28 log CFU/g for the thermal inactivation trials conducted at 50, 55, and 60°C, respectively, and ca. 7.11 ± 0.13, 7.13 ± 0.11, and 7.04 ± 0.08 log CFU/g, respectively, for compost with 40% moisture. For the same compost with 5 days of come-up time, the levels of *E. coli* O157 in the compost with 50% moisture were ca. 7.34 ± 0.77, 7.11 ± 0.07, and 7.47 ± 0.08 log CFU/g for thermal inactivation at 50, 55, and 60°C, respectively, and ca. 7.36 ± 0.44, 7.09 ± 0.77, and 7.14 ± 0.04 log CFU/g, respectively, in the compost with 40% moisture. The level of mesophilic background microflora that was present in the optimal compost mixture (C/N ratio of 25:1) was ca. 7.08 ± 0.15 log CFU/g, as enumerated by plating on TSA.

During the come-up time, there was a rapid decline in the surviving population of *E. coli* O157 just before the target composting temperature was reached (0 h) in all trials. With 2 days of come-up time, the *E. coli* O157 population in the compost with 50% moisture was reduced by ca. 4.64, 5.83 (enrichment positive), and 5.34 log reductions at 0 h of exposure to 50, 55, and 60°C, respectively, in comparison to population reductions of ca. 4.21, 4.90, and 3.86 log CFU/g, respectively, when the moisture of the compost was 40% at temperatures mentioned above (Tables 1, 2, and 3). The come-up time was extended to 5 days, the pathogen decline was still quicker in compost with 50% moisture with ca. 5.85, 5.47, and 6.07 (enrichment positive) log reductions at 0 h of exposure to 50, 55, and 60°C, respectively, than ca. 5.12, 4.80, and 5.52 log reductions, respectively, in compost with 40% moisture.

After the designated temperatures were reached, the compost mix with a 25:1 C/N ratio, 50% moisture, and 2 days of come-up time had ca. 5.60, 5.83 (enrichment positive), and 5.56 log reductions after 8, 4, and 1.5 h of exposure at 50, 55, and 60°C, respectively, and compared with ca. 5.94 (enrichment positive), 5.70, and 6.07 (enrichment positive) log reductions at the same sampling intervals and temperatures, respectively, with 5 days of come-up time (Tables 1, 2, and 3). The same compost with 40% moisture and 2 days of come-up time had ca. 5.55, 5.52, and 4.26 log reductions after 8, 4, and 1.5 h of exposure at 50, 55, and 60°C, respectively, and with 5 days of come-up time, ca. 5.72, 5.37, and 5.74 (enrichment positive) log reductions at the same sampling interval and temperatures, respectively.

In all trials of the compost with a C/N ratio of 25:1, greater reductions in *E. coli* O157 populations were observed in composts with 50% moisture than were observed in composts with suboptimal moisture (40%) during the same come-up times to the desired temperatures in the environmental chamber. With 2 days of come-up time, the pathogen was detectable by enrichment ranging from 72 h, 24 to 48 h, and 24 h at 50, 55, and 60°C, respectively, and 96 to 144 h, 48 to 120 h, and 48 to 72 h, respectively, at the same temperature with 5 days of come-up.

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**TABLE 1.** Thermal inactivation of *Escherichia coli* O157:H7 in fresh compost at 50°C under different conditions

<table>
<thead>
<tr>
<th>C/N ratio (moisture)</th>
<th>Treatment</th>
<th>Time (h)</th>
<th>log CFU/g at 50°C with heating time (h) of:</th>
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**Note:** Mean log CFU/g ± SD values with different rightmost uppercase letters differ significantly (P < 0.05) between two different treatments within the same moisture, C/N ratio, and come-up time within a column. Mean log CFU/g ± SD values with different lowercase letters differ significantly (P < 0.05) between two different come-up times within the same moisture and C/N ratio within a column for the same culture.

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**TABLE 2.** Thermal inactivation of *Escherichia coli* O157:H7 in fresh compost at 50°C under different conditions

<table>
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<tr>
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**TABLE 3.** Thermal inactivation of *Escherichia coli* O157:H7 in fresh compost at 50°C under different conditions

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time (Tables 1, 2, and 3). Overall, the pathogen survived longer in compost with low moisture (40%) and an extended come-up time (5 days) than in compost mix with 50% moisture and 2 days of come-up time. These results suggest heat adaptation of *E. coli* O157:H7 that may occur during an extended come-up time during the early phase of composting.

The difference in *E. coli* O157 survival between 2 and 5 days of come-up time in fresh compost with 50% moisture was significant (*P* < 0.05) at sampling times of 0, 2, 4, 6, and 8 h at 50°C, 0 h at 55°C, and 0 and 0.5 h at 60°C (Tables 1, 2, and 3). The difference in survival between 2 and 5 days of come-up time for the compost mix with 40% moisture was also significant (*P* < 0.05) at a sampling time of 0 h at 50°C and most of the sampling times at 60°C. Between composts with 40 and 50% moisture, *E. coli* O157 survival was significantly different (*P* < 0.05) from 0 to 4 h of the sampling times at both 55 and 60°C with 2 days of come-up time and at most of the sampling times at 50, 55, and 60°C with 5 days of come-up time.

**E. coli** O157 inactivation in fresh compost with C/N ratio of 16:1.** For 2 days of come-up time, the compost with 50% moisture was inoculated with *E. coli* O157:H7 at levels of ca. 7.23 ± 0.09, 7.18 ± 0.08, and 7.14 ± 0.06 log CFU/g for thermal inactivation at 50, 55, and 60°C, respectively, and the compost with initial moisture of 40% had ca. 7.20 ± 0.04, 7.12 ± 0.05, and 7.12 ± 0.08 log CFU/g of *E. coli* O157:H7, respectively. For the come-up time of 5 days, the same compost with 50% moisture was inoculated with *E. coli* O157 at levels of ca. 7.21 ± 0.07, 7.10 ± 0.08, and 7.11 ± 0.05 log CFU/g for the inactivation study at 50, 55, and 60°C, respectively, and the compost with 40% moisture had ca. 7.15 ± 0.07, 7.09 ± 0.04, and 7.13 ± 0.03 log CFU/g of *E. coli* O157:H7, respectively. The level of mesophilic background microflora that was present in this suboptimal compost mixture (C/N ratio of 16:1) was ca. 8.27 ± 0.08 log CFU/g as enumerated by plating on TSA.

The declines in the surviving populations of *E. coli* O157 were also rapid when the temperature of the compost reached the target level (0 h). The fresh compost mix with a 16:1 C/N ratio and a moisture content of 50% with 2 days of come-up time had ca. 3.32, 3.66, and 4.53 log reductions in *E. coli* O157 at 0 h of exposure to 50, 55, and 60°C, respectively, and ca. 3.03, 4.30, and 5.40 log reductions, respectively, with 5 days of come-up time (Tables 1, 2, and 3). Declines in *E. coli* O157 were even slower during the same target composting temperature when the moisture content of the compost was reduced to 40% with 2 and 5 days of come-up time.

After designated temperatures were reached, with 2 days of come-up time, the compost mix with a 16:1 C/N ratio and 50% moisture had ca. 5.38, 5.41, and 5.65 log reductions after 72, 12, and 4 h at exposure to 50, 55, and 60°C, respectively, and ca. 5.10, 5.61, and 5.71 (enrichment positive) log reductions at the same time and temperatures, respectively, with 5 days of come-up time (Tables 1, 2, and 3). For the same compost with 40% moisture, with 2 days of come-up time, *E. coli* O157:H7 was reduced for ca. 4.43, 3.69, and 4.13 log CFU/g after 72, 12, and 4 h of exposure to 50, 55, and 60°C, respectively, and with 5 days of come-up time, the reductions were ca. 4.38, 5.45, and 5.73 (enrichment positive) log CFU/g at the same sampling intervals and temperatures, respectively. When the come-up time was extended to 5 days, survival of *E. coli* O157 was extended in compost with both levels of moisture in compari-
### TABLE 3. Thermal inactivation of *O157:H7* in fresh compost at 60°C under different conditions

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>Treatment</th>
<th>MC (%)</th>
<th>Come-up time (days)</th>
<th>log CFU at 60°C with heating time (h) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
</tr>
</tbody>
</table>

- **C/N ratio**: The C/N ratio of fresh compost was 16:1 and 25:1.
- **MC (%)**: The moisture content of the compost was 40% and 50%.
- **Come-up time (days)**: The come-up time of the compost was 2, 4, 8, and 24 days.
- **log CFU at 60°C with heating time (h) of**
  - **0 h**: The logarithmic reduction of *O157:H7* was 0.
  - **0.5 h**: The logarithmic reduction of *O157:H7* was 0.5.
  - **1 h**: The logarithmic reduction of *O157:H7* was 1.
  - **1.5 h**: The logarithmic reduction of *O157:H7* was 1.5.
  - **2 h**: The logarithmic reduction of *O157:H7* was 2.
  - **4 h**: The logarithmic reduction of *O157:H7* was 4.
  - **8 h**: The logarithmic reduction of *O157:H7* was 8.
  - **24 h**: The logarithmic reduction of *O157:H7* was 24.

**Notes:**
- "+" indicates that the log CFU was greater than 3.
- "-" indicates that the log CFU was less than 0.
- "NS" indicates that the sample was not available.
- "NA" indicates that the sample was not detected.

- **C/N ratio of 25:1**: The survival of *O157:H7* in compost with 25:1 C/N ratio was more pronounced at 55°C, where *E. coli* O157 in compost with 50 and 40% moisture survived 4 and 5 days, respectively. However, survival of *E. coli* O157 was 3 days in compost with both types of moisture and 2 days of come-up time. At 60°C, survival of *E. coli* O157 in compost with 40% moisture was 2 and 3 days with 2 and 5 days of come-up time, respectively. Overall, pathogen survival was longer in the compost with the 16:1 C/N ratio than in the compost with a C/N ratio of 25:1 under most of the experimental conditions at the respective moisture levels and come-up times. For the compost (C/N ratio of 16:1) with low moisture and 5 days of come-up time, survival of pathogens was more extended than that in compost with 50% moisture or 2 days of come-up time.

- **Weibull modeling of thermal inactivation data**: Inactivation kinetics of *E. coli* O157:H7 at different temperatures and different composting conditions were fitted with a mixed Weibull model. Parameters of the Weibull model for different temperatures and composting conditions were given in Tables 4, 5, and 6. In the Weibull model, α was the model parameter (indicating the change in the ratio of the subpopulation resistant to stress). p was the shape parameter, and δ₁ and δ₂ were the decimal reduction times in subpopulation 1 (sensitive subpopulation) and subpopulation 2 (resistant subpopulation), respectively, that would rise due to stress.

All experimental conditions of composts with 25:1 C/N ratios were fit to the model with an *r*² value of >0.98. However, only 8 out of 12 experiments under the 16:1 C/N ratio compost trials were fit to the model with an *r*² value of >0.97. The remaining 4 trials which could not be modeled were probably due to more inactivation in the surviving populations of the pathogen during the come-up time than the other trials. At 50, 55, and 60°C, shape parameters were greater than 1 (p < 1) for all the tested conditions except at 50°C in compost with C/N ratio, moisture, and come-up time of 16:1, 40%, and 2 days, respectively (Tables 4, 5, and 6). In composts with an optimal C/N ratio (25:1), δ₁ and δ₂ values of the compost with 5 days of come-up time were greater than the corresponding δ₁ and δ₂ values of composting with 2 days of come-up time with the same moisture at all three temperatures. Similarly, the δ₁ and δ₂ values of the optimal compost with 40% moisture were greater than the respective δ₁ and δ₂ values at 50% moisture with the same come-up time. In composts with a suboptimal C/N ratio, similar trends were observed for δ₁ and δ₂ values between 40 and 50% moisture within 2 days of come-up time at 55 and 60°C.
The coefficient was obtained as follows:

\[
\ln(d) = 0.79 - (0.015 \cdot T) + (1.91 \cdot M) - (0.01 \cdot CN) + (0.04 \cdot H) - (0.03 \cdot T \cdot M) + (0.00001 \cdot T \cdot CN) - (0.002 \cdot T \cdot H) + (0.0003 \cdot M \cdot CN) - (0.13 \cdot M \cdot H) + (0.01 \cdot CN \cdot H) - (0.02 \cdot T \cdot H) - (0.11 \cdot M \cdot CN) - (2.48 \cdot M \cdot H) - (0.028 \cdot CN \cdot H) - (0.32 \cdot 43.20 \cdot 0.984)
\]

In the above equations, \( T, M, CN, \) and \( H \) represent temperature, moisture, C/N ratio, and come-up time, respectively. The \( r^2 \) values of regressions for equations 1 to 4 are 0.86, 0.96, 0.99, and 0.88, respectively. The high \( r^2 \) values indicate that parameters can be well described by the interaction response surfaces of four factors. The interaction terms of response surfaces

\[
\begin{align*}
\text{Value for mixed Weibull model parameter}^a & = (0.02 \cdot T \cdot H) + (0.17 \cdot M \cdot CN) - (1.31 \cdot M \cdot H) - (0.01 \cdot CN \cdot H) \\
\ln(\delta) & = 0.09 + (0.04 \cdot T) + (14.45 \cdot M) + (0.06 \cdot CN) + (0.75 \cdot H) - (0.20 \cdot T \cdot M) - (0.00004 \cdot T \cdot CN) - (0.001 \cdot T \cdot H) - (0.13 \cdot M \cdot CN) - (0.80 \cdot M \cdot H) - (0.002 \cdot CN \cdot H) \\
\ln(p) & = 18.19 + (0.24 \cdot T) + (31.51 \cdot M) + (0.10 \cdot CN) + (2.96 \cdot H) - (0.44 \cdot T \cdot M) + (0.001 \cdot T \cdot CN) - (0.02 \cdot T \cdot H) - (0.11 \cdot M \cdot CN) - (2.48 \cdot M \cdot H) - (0.028 \cdot CN \cdot H) - (0.32 \cdot 43.20 \cdot 0.984)
\end{align*}
\]

### TABLE 4. Parameters of mixed Weibull distribution of *E. coli* O157:H7 inactivation curves at 50°C

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>MC (%)</th>
<th>Come-up time (days)</th>
<th>Treatment</th>
<th>( \alpha )</th>
<th>( \delta_1 ) (h)</th>
<th>( \delta_2 ) (h)</th>
<th>( p )</th>
<th>4D value (h)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25:1</td>
<td>40</td>
<td>2</td>
<td>YC</td>
<td>5.48 ± 0.10</td>
<td>23.55 ± 1.32</td>
<td>97.94 ± 3.98</td>
<td>2.28 ± 0.16</td>
<td>44.4</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC</td>
<td>5.63 ± 0.09</td>
<td>23.25 ± 1.18</td>
<td>97.62 ± 3.52</td>
<td>2.28 ± 0.14</td>
<td>43.2</td>
<td>0.998</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>YC</td>
<td>5.30 ± 0.21</td>
<td>93.04 ± 9.64</td>
<td>1,260.60 ± 10.55</td>
<td>6.00 ± 2.31</td>
<td>118.8</td>
<td>0.985</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC</td>
<td>5.61 ± 0.33</td>
<td>22.20 ± 2.11</td>
<td>78.62 ± 10.91</td>
<td>2.65 ± 1.12</td>
<td>38.4</td>
<td>0.993</td>
</tr>
<tr>
<td>16:1</td>
<td>40</td>
<td>2</td>
<td>YC</td>
<td>5.21 ± 0.55</td>
<td>30.76 ± 4.65</td>
<td>124.07 ± 29.06</td>
<td>2.15 ± 0.44</td>
<td>59.04</td>
<td>0.976</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>YC</td>
<td>5.52 ± 0.2</td>
<td>21.91 ± 2.4</td>
<td>102.87 ± 9.91</td>
<td>1.69 ± 0.16</td>
<td>43.20</td>
<td>0.995</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 5. Parameters of mixed Weibull distribution of *E. coli* O157:H7 inactivation curves at 55°C

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>MC (%)</th>
<th>Come-up time (days)</th>
<th>Treatment</th>
<th>( \alpha )</th>
<th>( \delta_1 ) (h)</th>
<th>( \delta_2 ) (h)</th>
<th>( p )</th>
<th>4D value (h)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25:1</td>
<td>40</td>
<td>2</td>
<td>YC</td>
<td>5.48 ± 0.10</td>
<td>23.55 ± 1.32</td>
<td>97.94 ± 3.98</td>
<td>2.28 ± 0.16</td>
<td>44.4</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC</td>
<td>5.63 ± 0.09</td>
<td>23.25 ± 1.18</td>
<td>97.62 ± 3.52</td>
<td>2.28 ± 0.14</td>
<td>43.2</td>
<td>0.998</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>YC</td>
<td>5.30 ± 0.21</td>
<td>93.04 ± 9.64</td>
<td>1,260.60 ± 10.55</td>
<td>6.00 ± 2.31</td>
<td>118.8</td>
<td>0.985</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC</td>
<td>5.61 ± 0.33</td>
<td>22.20 ± 2.11</td>
<td>78.62 ± 10.91</td>
<td>2.65 ± 1.12</td>
<td>38.4</td>
<td>0.993</td>
</tr>
<tr>
<td>16:1</td>
<td>40</td>
<td>2</td>
<td>YC</td>
<td>5.21 ± 0.55</td>
<td>30.76 ± 4.65</td>
<td>124.07 ± 29.06</td>
<td>2.15 ± 0.44</td>
<td>59.04</td>
<td>0.976</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>YC</td>
<td>5.52 ± 0.2</td>
<td>21.91 ± 2.4</td>
<td>102.87 ± 9.91</td>
<td>1.69 ± 0.16</td>
<td>43.20</td>
<td>0.995</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) MC, moisture content.

\( b \) YC, young culture; LC, low-nutrient-adapted culture.

\( c \) Value are means ± SD. 4D value, time required to reduce 4 logs of *E. coli* O157 population at 55°C; \( r^2 \), adjusted \( r^2 \) value.

\( d \) —, data could not be fit to the mixed Weibull model.
showed that moisture \((M)\) and come-up time \((H)\) have significantly \((P < 0.05)\) high negative interactions for all four parameters.

**DISCUSSION**

During composting, the rate and level of heat generation by microbial activities and subsequent pathogen inactivation depends on various factors. In this study we investigated the effect of moisture, come-up time, and C/N ratio on the survival of \(E. coli\) \(O157:H7\) in fresh dairy compost at several composting temperatures in a controlled environment. Our results indicated that an optimal moisture level (50%) in fresh compost mix expedited \(E. coli\) \(O157:H7\) inactivation in comparison to the suboptimal moisture (40%) under various conditions and temperatures examined in the study. In this study \(E. coli\) \(O157:H7\) survived in fresh compost with 40% moisture for at least 12 and 5 days at 50 and 55°C, respectively, compared with 12 and 4 days in compost with 50% moisture at 50 and 55°C, respectively, under suboptimal composting conditions (C/N, 16:1, 5 days of come-up time). Under current guidelines, a moisture range of 50 to 60% is preferred; however, a range of 40 to 65% is also acceptable for composting (22). Based on our results, initial composting moisture needs to be defined in a much narrower range, since low initial moisture can extend the survival of pathogens.

Several studies have examined the impact of moisture level affecting pathogen inactivation during composting. Ceustersmans et al. (3) demonstrated that \(Salmonella enterica\) serovar Senftenberg strain W 775 was inactivated within 10 h of composting at 60°C with moisture varying between 60 and 65%; however, when the moisture content of the compost was reduced by 5%, the survival rate was increased by 0.50 log/h. The moisture content of the compost mixture affects temperature distribution within the compost heap (9, 20). When the moisture content of the compost mix is too high, conditions may turn anaerobic and the temperature of the compost heap will not rise, or the temperature rise will be very slow, thereby increasing the duration of the mesophilic composting phase. On the other hand, if the initial moisture level of the compost

**TABLE 6. Parameters of mixed Weibull distribution of \(E. coli\) \(O157:H7\) inactivation curves at 60°C**

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>MC (%)a</th>
<th>Come-up time (days)</th>
<th>Treatmentb</th>
<th>Value for mixed Weibull model parameterc</th>
</tr>
</thead>
<tbody>
<tr>
<td>25:1</td>
<td>40</td>
<td>2</td>
<td>YC</td>
<td>(\alpha = 4.97 \pm 0.30) (\delta_1 = 27.36 \pm 3.34) (\delta_2 = 74.51 \pm 8.72) (p = 2.41 \pm 0.43) (4 D = 48.96) (r^2 = 0.997)</td>
</tr>
<tr>
<td>25:1</td>
<td>50</td>
<td>2</td>
<td>YC</td>
<td>(\alpha = 5.33 \pm 0.21) (\delta_1 = 21.41 \pm 2.23) (\delta_2 = 71.67 \pm 6.56) (p = 2.14 \pm 0.25) (4 D = 41.28) (r^2 = 0.993)</td>
</tr>
<tr>
<td>16:1</td>
<td>40</td>
<td>2</td>
<td>YC</td>
<td>(\alpha = 4.63 \pm 0.25) (\delta_1 = 22.82 \pm 2.27) (\delta_2 = 71.69 \pm 7.43) (p = 1.70 \pm 0.18) (4 D = 52.8) (r^2 = 0.994)</td>
</tr>
<tr>
<td>16:1</td>
<td>50</td>
<td>2</td>
<td>YC</td>
<td>(\alpha = 5.20 \pm 0.36) (\delta_1 = 18.48 \pm 3.62) (\delta_2 = 77.06 \pm 14.09) (p = 1.65 \pm 0.29) (4 D = 43.2) (r^2 = 0.985)</td>
</tr>
<tr>
<td>16:1</td>
<td>50</td>
<td>5</td>
<td>YC</td>
<td>(\alpha = 5.28 \pm 0.18) (\delta_1 = 22.15 \pm 2.33) (\delta_2 = 71.67 \pm 6.56) (p = 2.14 \pm 0.25) (4 D = 41.28) (r^2 = 0.993)</td>
</tr>
</tbody>
</table>

* MC, moisture content.
* YC, young culture; LC, low-nutrient-adapted culture.
* Values are means ± SD. 4 \(D\) value, time required to reduce 4 logs of \(E. coli\) \(O157\) population at 60°C. \(r^2\), adjusted \(r^2\) value.
* —, data could not be fit to the mixed Weibull model.

**FIG. 1. Regression coefficients of four experimental factors, i.e., temperature, moisture, come-up time, and C/N, for 4 different model parameters (\(\alpha\), top left panel; \(\delta_1\), top right panel; \(\delta_2\), bottom left panel; \(p\), bottom right) in a mixed Weibull model.**
mix is too low, the microbial metabolic rate will be reduced for the microorganisms involved in composting, leading to slow temperature increases. In the present study, compost mixes with initial moisture levels of 40 and 50% were used. During temperature ramping in the environmental chamber, the compost mixes with optimal moisture (50%) would tend to lose more moisture in comparison to the compost with a suboptimal (40%) level. As a result, *E. coli* O157 in compost with optimal moisture may be inactivated more quickly due to development of more moist heat. In addition, pathogen populations in the compost with suboptimal moisture may have become adapted to heat stress compared to the pathogen levels in compost with an optimal moisture content. Gotaas (11) suggests that composts with optimal initial moisture content have a higher temperature zone that extends within most of the compost pile, with less stratification observed than when the initial moisture is suboptimal. Furthermore, differences in moisture levels could have arisen with the different treatments. Our moisture data revealed that the compost samples were drier at the end of 5-day come-up time than at the end of 2-day come-up time (data not shown), which may be the contributing factor stressing the pathogen and increasing its survival rate under thermal conditions of extended mesophilic composting.

In the present study, we found that at 55°C, *E. coli* O157:H7 survival was as short as 1 day and as long as 5 days depending upon the moisture level, come-up time, and C/N ratio. Despite this study being done under controlled conditions in a lab, the length of *E. coli* O157:H7 survival exceeded the EPA recommended guidelines of 3 days of composting at 55°C. Our results suggest inadequacy of time-temperature guidelines for composting when optimal composting conditions are not met. It is expected that field composting under similar conditions can further extend pathogen survival beyond the time limit observed in this study due to exposure to environmental variations.

In this study we found that when the composting process had a long mesophilic phase (5 days of come-up time) before it reached the thermophilic phase, *E. coli* O157:H7 was inactivated slowly during come-up time and survived for a longer time at specific composting temperatures in comparison to the situation where temperature rise was quick (2 days of come-up time). These results imply that an extended mesophilic phase of the composting process should be avoided to produce microbiologically safe compost. Previous studies have suggested that an extended mesophilic phase may allow the pathogens in compost to adapt to rising temperature, thereby surviving lethal temperatures by heat shock response induction (16, 23). Singh et al. (23) reported that *E. coli* O157:H7 heat shocked at 47.5°C survived for 5 h and 20 min at 55 and 60°C, respectively, compared with 3 h and 10 min, respectively, at the same temperature when not heat shocked. Lafond et al. (16) observed a cycle of appearance/disappearance of Gram-negative bacteria until day 32 of composting of duck excreta with a C/N ratio of 67.5:1. Since the composting process had a slow temperature rise, the authors suggest that Gram-negative bacteria developed heat resistance. In a field study, Shepherd et al. (21) found that heat-shocked *E. coli* O157:H7 survived 5 days at the bottom of the dairy compost, whereas non-heat-shocked culture survived for only 1 day. Apparently, to ensure complete inactivation of pathogens within the entire compost heap, it is necessary to take into consideration or monitor other stages of the composting process, such as the time (come-up time) required for the temperature of the compost heap to reach the thermophilic phase.

The carbon-to-nitrogen ratio is among one of the important factors affecting compost quality (10, 14). Generally, microorganisms use carbon for both energy and growth and available nitrogen for protein synthesis and reproduction. As a result, the generation of metabolic heat inactivates mesophilic pathogens during the thermophilic phase of the composting process. Initial C/N ratios of 25:1 to 30:1 are considered ideal for compost degradation, although C/N ratios of 20:1 to 40:1 are considered acceptable (19). In the present study, we found that compost with a suboptimal C/N ratio supported longer survival for *E. coli* O157:H7 than compost with the optimal C/N ratio within respective come-up times and moisture levels. Although, in our experimental setting, heat for microbial inactivation was produced by an environmental chamber rather than the self-heating from microbial metabolism, the difference in compost nutrient composition and microbial flora may be the reason for longer survival of *E. coli* O157:H7 in compost with a 16:1 C/N ratio. The importance of the C/N ratio during composting was highlighted in a field study by Huang et al. (13). That study showed that outdoor composting of pig manure under a wind-row system with a 30:1 C/N ratio (optimal) entered the thermophilic phase on day 3 of composting, indicating quick establishment of microbial activities in the composting pile, whereas about 7 days were required to reach thermophilic phase in compost with a C/N ratio of 15:1 (suboptimal). Such conditions are critical during composting, since composting under suboptimal conditions may facilitate microbial adaptation to a slow rise of temperature by mounting a heat shock response, thereby extending their survival. Also, in composting situations where the C/N ratio is high, e.g., >40:1, the temperature rise is slow and may not reach thermophilic phase, thereby affecting pathogen survival (16). Lafond et al. (16) found that compost with a C/N ratio of 67.5:1 had partial elimination of fecal streptococci, total coliform, and Gram-negative bacteria in comparison to the compost with a C/N ratio of 32.9:1, for which total coliform and fecal streptococci were undetectable after 6 and 12 days of composting, respectively. For small-scale composting, it may not be possible to control the C/N ratio of the compost mixture strictly within suggested limits. As a result, it would be difficult for the composting process to achieve time-temperature guidelines for pathogen inactivation. Also, if composting under such conditions is allowed to continue, then the composting process would take more time to mature due to an extended mesophilic phase and inadequate thermophilic phase (10, 13). However, the composting guidelines are not clear on such outcomes.

Historically, it has been believed that microorganisms in the population follow a first-order kinetic of thermal inactivation with some probability of dying for all (25, 26). Microbial communities are heterogeneous in nature; therefore, a survival curve having a shoulder and tail configuration is observed, deviating from linearity (23). In the present study, shape parameter *p*, which was >1 for the majority of the conditions, highlighted the nonlinear behavior of microbial populations in compost. Also, when microbial populations are exposed to...
stress, this characteristic further magnifies the variation in the populations (17).

In this study we used compost, which is a complex substance that additionally contributes to the microbial community variability as the heating medium. Both the δ₁ and δ₂ parameters of the model, which reflected sensitivities of the two populations in the microbial community to heat stress, were dependent on the physiological state of the microbial population and the physical state (percent moisture) of the compost. In a previous study (23), we also reported that microbial stress in the form of heat shock affected the values of these parameters. As discussed above, temperature is not the only factor that is important during composting. There are other factors that can affect the composting outcome, and those factors also need to be taken into consideration while studying microbial behavior during the composting process. Mathematical models that incorporate most of the factors that microbial cells are exposed to during composting will have high predictive strength (6). Based on our results, moisture was identified as one of the factors that affect pathogen survival during composting, with negative coefficients of correlation in the Weibull parameters δ₁ and δ₂ (Fig. 1) (P < 0.05). This means that inactivation of the pathogen would be quicker (lower δ₁ and δ₂ values) with the initial optimal moisture content (50%) of the compost than in the compost with suboptimal levels (40%). Additionally, the come-up time (heating process) in which the compost heap initially heats up had a positive correlation with parameters δ₁, δ₂, and p values (Fig. 1) (P < 0.05). This means that when the temperature rise is slow (5 days), then the inactivation of the pathogens is slow (higher δ₁, δ₂, and p) in comparison to that with optimal heating time (2 days). The parameter δ₂ was negatively correlated with temperature (P < 0.05), indicating that the higher the inactivation temperature of the composting processes, the quicker pathogen inactivation would be. Importantly, temperature is one of the critical factors during composting that adds selective pressure on microbial populations and differentiates the sensitive and resistant subpopulations. Clearly, in the present study, both moisture and come-up time contributed to the thermal resistance of the microbial population, in addition to temperature.

Although there was a correlation between the C/N ratio of the compost and the parameter α, the current experimental setup could not explain this. A similar study performed under field conditions would give more valuable information about this factor in relation to Weibull parameters.

It is obvious from the Weibull parameters (Tables 4, 5, and 6) that 4 D values for pathogen inactivation after 5 days of come-up time were greater than those after 2 days of come-up time for most of the treatments, suggesting the microbial population was more heat resistant when the temperature rise was slow. In the present study we found extensive tailing in the survival of E. coli O157:H7 during later stages of the inactivation study, indicating that some highly resistant subpopulations can survive the composting process. These surviving populations may later regrow under suitable conditions (9, 15).

Predictive equations that were developed in the present study can be used in predicting composting outcomes; however, this needs further validation since it is based on the experimental outcomes obtained in lab studies and there are many other environmental factors, such as seasonality, UV exposure, and precipitation, which may not be simulated correctly.

Our results clearly demonstrated that fresh dairy compost with 40% moisture supported better survival of E. coli O157 during active composting than the compost with 50% moisture. Come-up time was the most critical factor during our composting trials, with longer pathogen survival being observed for the composting condition which simulated a long mesophilic phase (5 days of come-up time) than for the one with a normal temperature rise (2 days of come-up time) regardless of the moisture level and C/N ratio. The thermal inactivation data fit well into the mixed Weibull model. Both experimental and modeling results suggest that microbial populations become adapted to composting temperatures when the temperature rise is slow or the composting was conducted under suboptimal conditions. Under certain conditions, e.g., and low-C/N compost mix with a longer mesophilic phase, E. coli O157:H7 survival exceeded 3 days at 55°C, the composting time-temperature recommendation in EPA guidelines, suggesting inadequacy of the guidelines for composting. Therefore, in order to ensure the microbiological safety of the composting process, the composting guidelines need to be refined further by taking into consideration suboptimal composting conditions.

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