




Use of screened dairy manure solids (SDMS) as composting amendment for carcase decomposition

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ABSTRACT

California is the largest agricultural producer in the United States and local dairy industry produces 21.5% of the national milk supply. There are 1470 dairies, 1789 million dairy cows and a total annual milk production of 18 million metric tons. The amount of dead cows to be disposed of is remarkable in intensive farming and it increases in periods of extreme weather events, such as drought in California. Composting of bovine mortalities is prohibited in California as a means of disposal of carcasses, and can only be done under an emergency declaration. Composting is an effective disposal method that can aid in carcase disposal, especially during an emergency. The objective of this study was to use screened dairy manure solids (SDMS) as the composting amendment for carcase decomposition. Our hypothesis was that temperatures would be sufficiently high and of sufficient duration to destroy most bacteria within the carcasses and that the leachate from the carcasses would penetrate less than one foot into the underlying soil. No significant amounts of leachate were noted in the collection pipes buried beneath either soil type. Total bacterial counts exceeded 1×10^6 CFU/ml in approximately 19% of the swab samples from the sample collection pipes. The sandy soil had higher bacterial counts than the clay soil. Results of these trials indicate that adult dairy cows can be successfully composted without significant impact on the nearby surrounding environment. The basic hypotheses have been verified by the simple and multiple regression and chi-square non-parametric test.

ARTICLE HISTORY

Received 13 September 2016
Accepted 28 November 2016

KEYWORDS

Carbon footprint;
composting; dairy;
environment; leachate;
screened dairy manure
solids

Introduction

California is the largest agricultural producer in the United States and dairy production is the single largest commodity. In 2014, the California dairy industry ranked first place in the US, with 42,337 million pounds, for the total milk production on farms, and represented 21.5% of the US total. There are 1470 dairies (1485 licenced dairies) and 1789 million dairy cows. In 2007, the California Regional Water Board implemented new waste discharge requirements due to concerns over animal agricultural pollution of the water supply. The waste discharge requirements have mandatory reporting, nutrient analysis of dairy animal waste and test wells for the sampling of ground water. This puts environmental impacts on an equal management priority with feed and disease management, and

milking hygiene. For the dairy producer, the new challenges in managing the environmental impact on a long-term, sustainable basis are still in their infancy. Long-term costs/impacts for producers are unknown so far.

The California dairy industry was impacted by unusually prolonged hot weather conditions during the summer of 2006. Starting in July 2006, a record was set for daytime temperatures exceeding 37°C in the San Joaquin Valley (SJV), a 300 by 50 miles wide mountain-walled valley that stretches from Sacramento to Bakersfield. The SJV is home to over 70% of California's 1.6 million dairy cows. The worst period was 14 consecutive days, with daytime highs that ranged from 39°C to 45°C. During this heat wave, even dairies with excellent cow cooling

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capabilities (shaded free stalls, sprinklers and evaporation fans) experienced losses from heat stress. In the aftermath, it was estimated that nearly 2% of California's dairy cows had died from heat related issues. This translated into approximately 40,000 additional cows dying during this period. In the central SJV, heat-related deaths were associated with a second, unforeseen event that caused a crisis in the management of carcasses from dead cows. One of two rendering plants available in the SJV for processing carcasses experienced mechanical failures and shut down for more than two weeks. In the initial stages of this crisis, there were over 1000 cow carcasses that could not be processed by renderers in the SJV, and there was no state mandated backup system in place for carcass disposal. In some cases, local regulations prevented burying animals or transporting mortalities to landfills. To address this crisis, seven counties declared a state of emergency to allow alternative disposal methods to be used (burial, composting and/or landfill). Through the hard work of local officials, rendering plants, and the emergency declaration, the disposal of carcasses was eventually brought under control.

Part of the long-term resolution of this problem will be to identify a safe, economical method for on-farm disposal of carcasses in the event that rendering capacity is overwhelmed. On-farm alternatives to rendering must address nuisances, such as flies and odors, as well as public health concerns related to pathogens and environmental impacts. Composting has been recommended by the USDA as a method for the disposal of large animal carcasses (USDA 2005).

All mortality composting processes utilise co-composting amendments to facilitate the process. Many composting amendments have been used successfully, including: composted manure, hay, saw dust, wood shavings, poultry litter, wheat straw, dry lot (corral) scrapings, and used bedding (Glanville and Trampel 1997, Keener et al. 2000). Central to the addition of the different types of organic matter used as amendments to compost carcasses is that they provide the following (Mukhtar et al. 2004): (i) sufficient carbon to achieve a carbon to nitrogen ratio of at least 25:1; (ii) sufficient porosity to allow gas exchange; (iii) a moisture content of 50% to 60%; (iv) sufficient dry matter to adsorb the leachate to prevent runoff. Composting amendments with these attributes will enable a first stage composting that achieves rapid decomposition (temperatures above 54 °C for 3 to 10 days) and a secondary decomposition with temperatures above 37.7 °C for several more days to weeks to complete and stabilise the process.

In states such as Iowa, Alabama, Virginia, Ohio, Georgia and New York, composting animal carcasses (including poultry and swine) is allowed. In other states, composting is only allowed under emergency declaration. Research data to address important safety and environmental concerns related to carcass composting would be helpful for local authorities responsible for protecting public and environmental health during emergencies (Di Renzo et al. 2015).

The objective of this study was to determine the extent to which ambient temperature, composting amendment available in the SJV, duration of composting and season would influence the temperatures within the composting piles using also the statistical methods of simple and multiple regressions. Our hypothesis was that temperatures would be sufficiently high and of sufficient duration to destroy most bacteria within the carcasses (>60 °C for 3–4 days) and that the leachate from the carcasses would penetrate less than a foot into the soil. The hypothesis of independence between the frequency of total bacterial levels >1 × 10⁶ CFU/ml in summer and winter trials has been verified separately for the Top and the Bottom of composting stacks through Chi-square non-parametric test.

Material and methods

Composting setup and design

Composting platforms

Four, 3.65 m long × 3 m wide × 0.6 m deep platforms were constructed (Figure 1). The floor and up to 30.48 cm of the sides of each platform was covered with 6 mm plastic sheets. Two of the platforms were filled with clay soil and the other two with sandy soil. Nine to ten PVC pipes (3.65 m long, 5 cm diameter) crossed the platform at approximately 20–32 cm below the top surface. Nine more pipes crossed the platform at approximately 43 cm below the top surface. There were 76 pipes (40 upper, 36 lower) in the 4 platforms. The buried pipes protruded approximately 30 cm on either side of the narrow width of the piles. The pipes were evenly distributed across the platform. Each pipe was perforated on the upper surface with 24, 1.5 cm diameter holes or an equivalent 1.5 cm slit. The ends of the pipes were closed with tight fitting caps. In Trial 1, each platform had a section for the composting carcasses (2.5 m) and a control section (1.2 m). There were two rows of six pipes under the carcass section of the pile and two rows of 4 pipes under the control section of the pile. Each pile also had three, 8 cm diameter, perforated drain pipes in the upper row of pipes that

remained open during the study. Two of these were under the carcasses and one under the control area. During Trial 2, several of the carcasses were placed in the centre of the platform so that the control sections were on either end of the platforms.

Trial location

The platforms were located in a dry, wastewater storage pond, constructed to meet dairy specifications. The area was fenced and a rodent control programme was used.

Composting amendment

Four loads (20 m³) of SDMS, screened dairy manure solids, were used as the co-composting material. The manure had been stacked at the manure separator on a local dairy. This material would have been further dried and used for bedding in a free stall dairy barn. Approximately, 3 m³ of SDMS were placed on each platform prior to loading the carcasses. This formed a layer approximately 45 cm deep. After the carcass was placed on the platform, enough SDMS was placed over the carcass to provide approximately 50 cm of covering. When the piles sunk as the carcasses decayed, additional SDMS was placed on the piles on two or three occasions during the first 60 days of each trial.



Figure 1. Platforms used to compost adult dairy cows, using dried solids separator manure as a composting amendment. Pipes protruding from wood sides allowed sampling of leachate that could penetrate the soil beneath the carcasses composting in recycled manure. This is from the summer trial (Trial 1). The box is marked to show the placement of the cow on the right, with the control portion on the left. The composting pile is from the end of the composting period at 137 days, and is partially dug-up to expose the remains of the carcass from an adult dairy cow.

Carcasses

Opened carcasses were obtained from the California Animal Health and Food Safety Laboratory (CAH&FS) in Tulare. Small tissue samples for diagnostic purposes had been removed from the carcasses and the remaining viscera and fluids collected in barrels. The carcasses were placed on the platforms and the viscera and fluids poured into the open space of the abdomen. During Trial 2, two carcasses were composted without opening the carcasses. At the conclusion of the study, the remaining bones and tissues were delivered to the diagnostic laboratory for disposal.

Composting period

Composting of the carcasses in Trial 1 began on July 1, 2008 when four carcasses were placed on the platforms and covered. Trial 1 ended on 16 November 2008 (137 days) when the carcasses were uncovered and the remaining bones collected. For Trial 2, two carcasses (1–2) were started on 7 December 2008, two (3–4) on 3 January 2009 and the final two (5–6) on 6 January 2010. Carcasses 1–4 were uncovered on 10 May 2009 (143 days for 1–2; 138 days for 3–4). The remaining two carcasses (5, 6) were unearthed on 25 May 2009 (140 days).

Data collection

Temperature recordings within the carcasses

In Trial 1, each of the four carcasses had three temperature recorders placed within the carcass. These were sealed probes, Optic Stowaways, set to record data at five minute intervals throughout the composting period (Onset Computer Corp., Bourne, MA 02532). One recorder was placed in the back of the mouth (head), one was placed in the rear of the abdominal cavity (rear) and one was placed in the thoracic or chest cavity (fore). In Trial 2, the temperature recording devices were placed near the carcasses (not inside) in two locations after the start of the trial as the recorders were being repaired when the trial started.

Manual temperature recordings within the composting piles

Using a 1 m-long temperature probe attached to a digital reader, the temperatures within the composting piles were recorded during the composting period. The temperatures were taken above and below the carcasses and at similar locations in the control portion of the piles. Temperatures were recorded on 27 time

points (days) during the 137 days in Trial 1 and 26 time points during the 143 day duration of Trial 2.

Climatic information

Information on precipitation and temperature for the Visalia area from the Western Regional Climate Centre, Desert Research Institute in Reno, NV was used for the trials. Visalia is located about 10 miles north of the VMTRC composting site.

Sampling for culture

Each of the forty 5 cm diameter pipes were cultured five times during Trial 1 (days 1, 7, 14, 28 and 83) and four times during Trial 2 (days 7, 36, 49 and 63). To sample the pipes, the caps were removed and PBS soaked 4×4 swabs were pushed 3 m into the pipes and pulled out. The swabs were taken directly to the Dairy Food Safety Laboratory for microbial analysis. Using SBA (Sheep blood agar) and MacConkey agar, the bacteria were classified as total bacteria, Gram-negative bacteria, lactose-positive bacteria or lactose-negative bacteria. On the final days of the study when the carcasses were uncovered, 6 samples of the composting materials were taken from each pile. Four samples were taken from above and below the remaining bones and two from the control portion of the pile.

Statistical approach and verification

The research questions and the hypothesis advanced need the development of a static model which enables us to include the independent variables, and precisely the Ambient temperature (AT), the Precipitations limited to winter (P) and the Time elapsed (TE – as proxy of composting amendment effects) – to explain their effects on the Inside temperature (IT) of the piles (dependent variable). Consequently, single and multi-regression models have been established for comparing those effects on the IT in summer, and respectively in winter, to capture the differences if there are.

Linear Regression is the simplest relation that connects a dependent variable Y with an independent variable X :

$$Y = \alpha + \beta X \quad (1)$$

As it is known α and β are the coefficients that minimise the error $\sum_{j=1}^N (\alpha + \beta X_j - Y_j)^2$, namely the sum of the square of single point error. The parameter β is the one of greatest interest since it expresses the intensity of the effect exerted by the independent variable on the dependent (apart scale factors).

Should the error be not satisfactory, a multivariate linear regression model has been used which operates in the same way. The dependent variable Y is a function of one or more independent variables and one or more control variables $X_{R, R=1, \dots, n}$. The formula is:

$$Y = \alpha + \sum_{i=1}^K \beta_i X_i \quad (2)$$

Remark that at least $K+1$ data must be associated to different (X_1, \dots, X_K) points otherwise no solution can be found. Multiple linear regression estimates the parameters α e β_k (for $k=1, \dots, K$) corresponding the hyperplane that minimises the sum of squared prediction errors (Corbetta et al. 2001, p. 200). The α parameter expresses the predicted value of Y when all the regressors or predictors X_k are zero. β_k represents the average variation of Y value produced by each unit change of the regressor x_k when the assumed value of all the other regressors is constant (Corbetta et al. 2001, p. 201).

The verification of the model begins with a simple linear regression between the data of Ambient temperature (AT) as independent variable and the average of the Inside temperature (IT) of the composting piles as dependent variable during the Summer (four stacks) and Winter (two first stacks). For summer, the expression obtained is $IT = 2.1435 AT + 5.2593$ with a good regression coefficient $R^2 = .7663$ which explains more than 76% of the phenomenon, suggesting the existence of a positive correlation between the two variables. As winter the calculated equation is $IT = -0.9879 AT + 65.11$, and the $R^2 = .438$ explains a little less 50% of the variability. It is notable that the sign of the coefficient β of AT predictor is negative, indicating that AT and IT vary in the opposite direction and the correlation between them is inverse. It needs to identify further significant predictors to improve the fitness of the regression.

The hypothesis here formulated is that both in summer and in winter, the Time elapsed (TE), total days from the beginning of the composting process to each successive IT measurement, could have a certain influence as independent variable and, limited to winter, also Precipitations (millimeters of ambient water fallen in Visalia as rain in the three days preceding the IT measurement).

Applying the multiple regression analysis on summer and winter data with a stepwise introduction of one more variable, that is Time elapsed, both predictors demonstrated to be significant because each of them has a correlation with the criterion at least of $|0.2|$, but they have also between themselves a high

Pearson correlation coefficient (summer, $r = -.781$; winter, $r = .719$). The analysis of the collinearity statistics shows that the Tolerance and the VIF, for summer and winter, are a little out of the range. For both the predictors, the Tolerance is a bit less the threshold (> 0.5) and the VIF is a little more than 2. Limited to winter, it has been considered also a further predictor that is Precipitations (PR). The Pearson partial correlation coefficient between PR and the criterion IT is very low ($r = -.036 < |0.2|$) suggesting that PR is a casual variable, notwithstanding in the first instance this predictor is going to be considered in the multiple regression analysis. In the Collinearity diagnostics, Variance proportions are not exactly in the rule for AT vs TE and also for TE vs. PR, but the Eigenvalues are significantly > 0 and the 'Condition indexes' are much less than the threshold of 15 so that it is reasonable to maintain in the analysis all the variable (Barbaranelli 2006; Chiorri 2010).

Beginning with summer, two models have been found. The first model is the same described above; the second model, which includes the further predictor TE, improved the determination coefficient R^2 to .915 (Table 1) and the equation estimated is $IT = 0.963 AT - 0.183 TE + 43.998$. The adjunct variable explains a 14.87% more of the initial variability but its sign is negative. In summer, the effect of TE (rising variable) on the IT of piles is always subtractive, while that of AT is in a first period additive, as the air temperature increases, but becomes subtractive when in the successive times the AT decreases.

The calculated equation for winter is $IT = -0.894 AT - 0.120 TE + 64.806$, $R^2 = .464$. With the addition of the TE predictor the fitness of the linear function improves of 2.6%. As time elapsed, the effect of TE predictor (rising variable) and the AT ones (decreasing variable) is all the time subtractive of the pile internal temperature (IT). The comparison of the two equations for summer and winter reveals that they are greater in

summer than in winter and therefore the effects of their variability are in winter a bit attenuated.

Implementing for summer the stepwise criterion to the second model to exclude those variable with a probability-of-F-to-enter $\leq .050$ or a probability-of-F-to-remove $\geq .100$, the results suggest the elimination of one of the two predictors suspected to be collinear and, in particular, Ambient temperature though its insertion improved the determination coefficient as noted above. The more convincing conclusion is that, in the specific environmental conditions of California, both variables Ambient Temperature and the Time Elapsed are crucial and able to explain the variability of the internal temperature of the stacks (Yu et al. 2008). TE is a proxy of the normal evolution of the aerobic digestion process. From this is apparent that these predictors influence the effectiveness and the speed of the composting process in California. The choice of one of them as the unique explanatory variable is oscillating from stack to stack. An explanation of the phenomenon can be given assuming that: (a) the thickness of the clay layer in the two stacks with abnormal behaviour is lower than that of the other; (b) the clay used has a different level of humidity; (c) the composting amendments used have a different water content, the compost in theory should have a standardised moisture; (d) the weight of carcasses is different; (e) the sandy substrate at the base of the stack is more or less wet. These little casual variants may have a greater effect on one of the two predictors, from time to time, and hence support alternative choices.

For winter, forcing the regression to consider three predictors, rather than two, by the Enter method, one single model is obtained with an $R^2 = .513$, and a predictive equation $IT = -0.861 AT - 0.031 PR - 0.027 TE + 66.01$ which explains more than 50% of the variability and improves the R^2 of the preceding regression with only two predictors by 5%. The influence of the other two variables is negative too: varying the

Table 1. Results of the Multiple Regression: Models Summary.

	R	R ²	Adjusted R ²	Std. Error of the Estimate	R ² Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
Summer										
1	.924 ^a	.855	.849	4.61471	.855	164.530	1	28	0.000	
2	.956 ^b	.915	.908	3.59850	.060	19.047	1	27	0.000	0.960
Winter										
1	.679 ^a	.461	.438	3.618	.461	20.51	1	24	0.0001	
2	.682 ^b	.465	.418	3.683	.004	0.162	1	23	0.691	
3	.716 ^c	.513	.447	3.59	.049	2.2	1	22	0.152	1.494

Authors' elaborations.
 Legenda: Predictors: (Constant);
^aSummer TE; Winter AT;
^bTE, AT;
^cTE, AT, PR.

Ambient temperature, the Precipitations and the Time elapsed in the same direction, the Inside temperature of piles reduces itself. Their partial and global effects are in general subtractive. The limited explaining capability could also depend on a bad choice of experimental data. For winter, the technical problem of the detection of the internal temperature, due to the hard soil, makes the data difficult to compare with summer. In particular, the first period (up to the 33 days from the beginning) presents a pattern which does not conform to that of the summer as is on the contrary the case of the following period albeit with weaker coefficients.

The hypothesis of independence between the frequency of total bacterial levels $>1 \times 10^6$ CFU/ml in summer and winter trials has been verified separately for the Top and the Bottom of composting stacks through Chi-square non-parametric test. The aim of the chi-square (χ^2) is to verify if the frequencies of well observed values fit to the theoretical frequencies of a predetermined distribution of probability. Denoting by f_o the observed frequencies and by f_e the expected frequencies under the hypothesis of independence, the mathematical relationship is:

$$\chi^2 = \sum \frac{(f_o - f_e)^2}{f_e} \quad (3)$$

χ^2 will be zero in the case of perfect independence between data while will be all the greater if the observed frequencies will move away from the assumption of independence. In other words, it measures the strength of the relationship between two variables (Corbetta et al. 2001, p. 130), but the χ^2 value is directly related to the number of cases. In order to overcome this problem, it has been applied the V index (Cramer's V) that varies values between 0 (independence) and 1 (perfect relationship):

$$V = \sqrt{\frac{\chi^2}{N(k-1)}} \quad (4)$$

where k is equal to the number of modalities of the variable with the least number of them (Corbetta et al. 2001, p. 131).

From the non-parametric analysis, it results that chi square index calculated on the frequencies of total bacterial levels $>1 \times 10^6$ CFU/ml in summer and winter trials is $\chi^2 = 0.094$ for the top of the piles and respectively $\chi^2 = 1.128$ for the bottom. The comparable Cramer's indexes V is 0.153 for the top and resp. 0.531 for the bottom of piles. These results express the low probability that in summer and in winter the total bacterial frequencies at the top of the piles are dependent

while those at the bottom have a medium probability to be each other influenced.

The chi-square test allows to compare the χ^2 value with a theoretical value of reference (Vt) calculated on the percentile values of the distribution of the χ^2 random variable with v degrees of freedom. If Vt is greater than the χ^2 value then the observed values are not due to the randomness but they are associated each other, while in the opposite case what has been observed is simply due to the randomness. Since the above values are very low and in any case lower than the critical level $\chi^2_{0.95} = 7.81$ for 3 degrees of freedom, the hypothesis H_0 of independence between summer and winter variables in the frequency of the distribution of the bacterial load at the top and at the bottom of the piles examined is accepted.

Results

Composting leachate

The two 5 cm pipes often contained clear, condensate water that occurred after the composting process was started. During the composting period, visible amounts of leachate from the composting carcass piles was not seen in the large 8 cm collecting pipes located at about 20 cm below the soil surface of the platforms and at least 45 cm below the carcasses. Water did condense in the capped, smaller pipes, particularly during Trial 1 when the temperatures were higher than Trial 2. The water in amounts of less than 200 ml was noted as clear liquid on the sampling dates when the pipes were opened for sampling. During Trial 2, similar amounts of liquid were found and it was more common to find brownish liquid in the 5 cm pipes. This occurred at random locations. Most of the small pipes contained clear, condensate water, however, in smaller amounts than during Trial 1.

Bacteria in leachate

For Trial 1, a total of 380 swab samples from the collecting pipes (5 cm diameter) were cultured. Of those, 200 came from under the carcasses and 180 came from under the control section of the composting pile. The primary isolates from the microbial analysis were Gram-positive. Gram negative bacteria comprised only 10% of bacterial flora and the majority of these were lactose-negative on MacConkey agar.

The two platforms with clay soil had 38 pipe swab samples with $>1 \times 10^6$ total CFU bacteria per ml (top pipes 25; bottom pipes 13), whereas the sandy soil samples had 37 results that were $>1 \times 10^6$ CFU total

Table 2. Summer trial (Trial 1).

	Row	Day 0	Day 7	Day 14	Day 28	Day 83	Total
Stack 1	Top	0	6	5	3	0	14
Stack 1	Bottom	0	4	2	2	0	8
Stack 2	Top	0	8	0	0	0	8
Stack 2	Bottom	0	9	0	0	0	9
Stack 3	Top	0	8	1	1	1	11
Stack 3	Bottom	0	5	0	0	0	5
Stack 4	Top	0	5	2	0	0	7
Stack 4	Bottom	0	7	5	1	0	13
Total		0	52	15	7	1	75

Frequency of total bacterial levels $>1 \times 10^6$ CFU/mL over time during composting for samples isolated from collection pipes below the carcass. Results show the first 83 days of the 137 day composting period, when pipe sampling was terminated. Stacks 1 and 3 are clay; Stacks 2 and 4 are sand.

Table 3. Winter Trial (Trial 2).

	Row	Day 8	Day 37	Day 50	Day 64	Total
Stack 1	Top	0	5	5	6	16
Stack 1	Bottom	1	2	1	2	6
Stack 2	Top	1	4	1	0	6
Stack 2	Bottom	2	2	0	0	4
		Day 10	Day 23	Day 37		
Stack 3	Top	1	2	4		7
Stack 3	Bottom	2	0	0		2
Stack 4	Top	1	4	2		7
Stack 4	Bottom	0	0	2		2
						50

Frequency of total bacterial levels $>1 \times 10^6$ CFU/mL over time during composting for samples isolated from collection pipes below the carcass. Results show the first 64 days of the 143 day composting period, when pipe sampling was terminated. Stacks 1 and 3 are clay; Stacks 2 and 4 are sand.

Top indicates sample collection pipes placed approximately 20 cm below the soil surface with 10 collection tubes laid across the composting box for sampling purposes.

Bottom indicates sample collection pipes placed approximately 45 cm below the soil surface with 9 collection tubes used for sampling.

bacteria per ml (top pipes 15; bottom pipes 21) (Table 2). No pipe samples had $>1 \times 10^6$ CFU total bacteria per ml on Day 0. Almost 70% of these samples were collected on Day 7 of the trial and 89.3% that exceeded $>1 \times 10^6$ total CFU were found by Day 14. Of these samples with $>1 \times 10^6$ total CFU per ml, 62 (31% of 200) came from under the carcass portion of the platform (top pipes 33, bottom pipes 29) and 13 (7.2% of 180) came from under the control portion of the platform (top pipes 7, bottom pipes 6) (Table 3).

For Trial 2, a total of 262 swab samples from the collecting pipes (5 cm diameter) were cultured. Of those, 144 came from under the carcasses and 118 came from under the control section of the composting pile. The primary isolates from the microbial analysis were again Gram-positive, with approximately 10% being Gram negative, lactose-negative bacteria. The two platforms with clay soil had 31 pipe swab samples with $>1 \times 10^6$ CFU total bacteria per ml, whereas the sandy soil samples had 19 results that

were $>1 \times 10^6$ CFU total bacteria per ml (Table 3). Similar numbers of samples with $>1 \times 10^6$ CFU per ml were found on Days 36, 49 and 63 of the composting period. Of the samples with $>1 \times 10^6$ total CFU per ml, 36 came from under the carcass portion of the platform (top pipes 26, bottom pipes 10) and 14 came from under the control portion of the platform (top pipes 10, bottom pipes 4) (Table 4).

On completion of the composting period (Day 143, 128), 24 samples of the composting material were cultured for bacteria. Of these samples, none had $>1 \times 10^6$ total CFU per ml of bacteria.

Carcass and compost pile temperatures

For Trial 1, temperatures recorded within the four carcasses at all locations (rear, fore and head), exceeded 60°C on average for 35 days, (range 1 to 52 days). Carcass temperatures at all locations exceeded 65°C for an average of 22 days, (range 0 to 41 days), and $>70^\circ\text{C}$ for 2.5 days (range 0–11 days). All the recorders in the rear of the abdomen exceeded 60°C for >27 days; the fore or thoracic location exceeded 60°C for 20 days, with the exception of animal 4, which was for one day. The head location exceeded 60°C for at least 29 days (Table 5).

During Trial 1, pile temperatures that were assessed manually with a three foot probe were taken on 27 different days. A total of 216 readings were taken around the carcasses and 108 readings were taken in the control portions of the piles. The first temperatures were taken on day 7 of the composting period and all the piles exceeded 65°C at this time (Table 6 and Figure 2). The duration of pile temperature exceeding 60°C below the carcasses was from 43 to 65 days, and above the carcasses the temperature exceeded 60°C from 32 to 57 days. The temperatures rose above 65°C by day 4, after the beginning of the composting in three of the four piles, and by day 7 in the remaining pile. In the control portion of the piles, the temperature within the pile exceeded 60°C from 4 to 23 days above the carcass level and from 4 to 21 days below the carcass level.

For Trial 2, twelve continuous recording temperature devices were again used. Of the twelve recorders, seven locations near four of the carcasses exceeded 60°C. The duration for temperatures that exceeded 60°C was 2–18 days. Five locations did not exceed 60°C during the trial and no location exceeded 65°C during the winter trial (Table 7). It should be noted that during the winter trial, the recorders were placed next to the carcass, not within the carcasses as in Trial 1.

Table 4. Summer trial (Trial 1) and Winter trial (Trial 2).

Stack	Top Pipes Carcase Section		Bottom Pipes Carcase Section		Top Pipes Control Section		Bottom Pipes Control Section	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Stack 1	12	10	7	3	2	6	1	3
Stack 2	6	4	6	3	2	2	3	1
Stack 3	9	7	5	2	2	0	0	0
Stack 4	6	5	11	2	1	2	2	0
Total	33	26	29	10	7	10	6	4

Frequency of samples $>1 \times 10^6$ CFU/mL between control portion of the pile and carcase portion of the pile for samples isolated from collection pipes below the carcase. Stacks 1 and 3 are clay; Stacks 2 and 4 are sand.

Top indicates sample collection pipes placed approximately 20 cm below the soil surface.

Bottom indicates sample collection pipes placed approximately 45 cm below the soil surface.

Table 5. Trial 1, summer – temperatures measured with Optic Stowaway data loggers placed near the carcasses.

	Data Logger Placement	Days Above 140 °F	Days Above 131 °F ^b	Days Above 104 °F ^c
Stack 1	Rear ^a	42	69	134
	Fore ^a	20	29	120
	Head ^a	46	72	134
Stack 2	Rear	27	42	89
	Fore	42	52	95
	Head	52	63	117
Stack 3	Rear	51	70	125
	Fore	ND	ND	ND
	Head	47	70	115
Stack 4	Rear	33	49	125
	Fore	1	7	71
	Head	29	43	125

Data loggers were set to record temperatures every 5 minutes. Daily temperatures considered for the indicated cut-offs were maintained for entire 24 hour periods.

^aTemperature recorder placement: rear of the abdominal cavity (rear), thoracic or chest cavity (fore), back of the mouth (head).

^bEPA has classified Class A bio-solids as having a sustained temperature of 131 °F/55 °C for 3 days.

^cEPA has classified Class B bio-solids as having a sustained temperature of 104 °F/40 °C for 5 days.

For Trial 2, the temperatures taken with the manual probe within the composting piles were taken on 26 sampling days during the 137 day composting period for Stacks 1 and 2 and for 22 sampling days on the other four stacks. The left and right-hand temperatures represent the control areas of the stacks, whereas the centre temperatures represent the carcase portion of the stacks. In 31 of the 36 compost pile areas probed for temperature, the temperature exceeded 60 °C for at least 1 day (range 0 to 51 days) (Table 8 and Figure 3). The average number of days in the carcase locations, when the temperature exceeded 60 °C, was 19.5 days (range 0 to 38 days) below the carcase and 31.3 days (range 15 to 35 days) above the carcase. In the control locations, the temperature exceeded 60 °C on average for 15.3 days (range 0 to 35 days) below the carcase level and 23.8 days (range 1 to 39 days) above the level of the carcase. It should be noted that for 16 days (17 December 2008 through 2 January 2009)

Table 6. Trial 1, summer – probe temperatures (°F) taken manually in the composting stacks with a three foot probe.

Stack	Location	Days Above 140 °F	Days Above 131 °F ^c	Days Above 104 °F ^d
1	Control Below ^a	4	33	67
	Control Above	4	29	64
	Cow area Below ^b	54	72	135
	Cow area Above	36	43	89
2	Control Below	24	34	67
	Control Above	23	33	67
	Cow area Below	65	70	107
	Cow area Above	57	70	100
3	Control Below	6	40	81
	Control Above	21	36	81
	Cow area Below	60	84	107
	Cow area Above	44	69	100
4	Control Below	21	43	81
	Control Above	5	36	64
	Cow area Below	43	57	107
	Cow area Above	32	43	89

^aTemperatures were taken above and below the carcase level. The area adjacent to the carcase is indicated as the control portion of the piles.

^bTemperatures taken above and below the carcasses in the carcase portion of the pile.

^cEPA has classified Class A bio-solids as having a sustained temperature of 131 °F/55 °C for 3 days.

^dEPA has classified Class B bio-solids as having a sustained temperature of 104 °F/40 °C for 5 days.

the probe temperatures were not taken. Thus, temperatures may have been higher than 60 °C for more than 60 days in all locations, whether in the carcase or control portion of the stacks.

Rainfall

More total rain and rainfall events occurred during Trial 2 compared to Trial 1, (Figure 4). These events did not appear to influence the bacteria counts within the pipe samples. However, the temperatures within the composting material did increase, following each significant rainfall event. Three rainfall events occurred during Trial 1. Between October 17 and 21 (Days 102–106), there was 2.39 cm of rain. On 27th October (Day 112) there was 1.93 cm of rain and on 4th November (Day 120) there was 0.74 cm of rain. A slight, but not uniform rise in temperature of approximately 5 °C was noted in the stacks. Trial 2 occurred during the winter and, as



Figure 2. Trial 1, Summer: Probe temperatures ($^{\circ}\text{C}$) manually taken above and below carcasses by probing the pile with a 3-foot long temperature probe during the composting period.

Table 7. Trial 2, winter – temperatures measured with Optic Stowaway data loggers placed near the carcasses.

	Data Logger Placement	Days Above 140 $^{\circ}\text{F}$	Days Above 131 $^{\circ}\text{F}^{\text{b}}$	Days Above 104 $^{\circ}\text{F}^{\text{c}}$
Stack 1	Right ^a	0	23	117
	Left ^a	0	19	117
Stack 2	Right	1	19	117
	Left	18	45	117
Stack 3	Right	2	17	69
	Left	4	27	117
Stack 4	Right	0	0	117
	Left	0	0	117
Stack 5	Right	0	7	41
	Left	7	27	85
Stack 6	Right	9	30	98
	Left	3	37	117

Data loggers were set to record temperatures every 5 min. Daily temperatures considered for the indicated cut-offs were maintained for entire 24-h periods.

^aIn Trial 2, the temperature records were placed near the carcasses in two locations after the start of the trial, as the recorders were being repaired when the trial started.

^bEPA has classified Class A bio-solids as having a sustained temperature of 131 $^{\circ}\text{F}$ /55 $^{\circ}\text{C}$ for 3 days.

^cEPA has classified Class B bio-solids as having a sustained temperature of 104 $^{\circ}\text{F}$ /40 $^{\circ}\text{C}$ for 5 days.

anticipated, there were several rainfall events (Figure 4). In Trial 2, Stacks 1 and 2 had significant increases in pile temperatures after rainfalls. In some cases, after the rainfall occurred early in the composting period (first 60 days), the temperatures increased by 5 $^{\circ}\text{C}$ to 15 $^{\circ}\text{C}$ and then returned to the 60 $^{\circ}\text{C}$ temperature range. Rainfall later in the composting period (after 90 days) was followed by increases in the stack temperatures, but not to the extent that was noted in the earlier composting periods. In the six days preceding the sampling of the pipes on 13th January, there was 4.52 cm of rain. At this sampling, 17 samples (23%) contained more than 10⁶ CFU total bacteria. On two other sampling dates, there were 14 (19%) and 18 (24%)

Table 8. Trial 2, winter – probe temperatures ($^{\circ}\text{F}$) taken manually in the composting stacks with a three foot probe.

Stack	Location	Days Above 140 $^{\circ}\text{F}$	Days Above 131 $^{\circ}\text{F}^{\text{a}}$	Days Above 104 $^{\circ}\text{F}^{\text{b}}$
1	Left Below	1	78	118
	Left Above	5	29	118
	Cow area Below	0	44	118
	Cow area Above	34	78	118
	Right Below	22	36	118
	Right Above	12	18	112
2	Left Below	20	63	126
	Left Above	15	54	122
	Cow area Below	38	47	126
	Cow area Above	22	100	126
	Right Below	1	44	126
	Right Above	14	22	126
3	Left Below	35	50	109
	Left Above	34	46	113
	Cow area Below	18	29	109
	Cow area Above	15	35	103
	Right Below	1	24	110
	Right Above	11	17	102
4	Left Below	24	26	106
	Left Above	35	50	109
	Cow area Below	24	28	103
	Cow area Above	22	33	110
	Right Below	0	36	109
	Right Above	13	28	109
5	Left Below	30	36	109
	Left Above	28	46	109
	Cow area Below	3	36	110
	Cow area Above	32	56	111
	Right Below	0	17	110
	Right Above	16	72	109
6	Left Below	0	0	111
	Left Above	1	106	111
	Cow area Below	0	29	111
	Cow area Above	35	61	110
	Right Below	21	46	111
	Right Above	39	49	103

Temperatures were taken above and below the carcasses level. The area adjacent to the carcass is indicated as the left and right-hand portion of the piles.

^aEPA has classified Class A bio-solids as having a sustained temperature of 55 $^{\circ}\text{C}$ for 3 days.

^bEPA has classified Class B bio-solids as having a sustained temperature of 40 $^{\circ}\text{C}$ for 5 days.

samples with this level of growth and neither was preceded by a rainfall event.

Seasonal temperature

Both the maximum and minimum average daily temperatures were higher in Trial 1 (July–November) compared to Trial 2 (December–May). The average daily air temperature during Trial 1 was 30.4 $^{\circ}\text{C}$ (37.8 $^{\circ}\text{C}$ –14.4 $^{\circ}\text{C}$) maximum and 15.5 $^{\circ}\text{C}$ (21.7 $^{\circ}\text{C}$ –5 $^{\circ}\text{C}$) minimum. The average daily air temperature during Trial 2 was 16.4 $^{\circ}\text{C}$ (27.2 $^{\circ}\text{C}$ –5 $^{\circ}\text{C}$) maximum and 6.5 $^{\circ}\text{C}$ (12.2 $^{\circ}\text{C}$ –1.7 $^{\circ}\text{C}$) minimum.

Duration and extent of composting

On Day 139 after the composting began for the summer trial, the remaining parts of the carcasses were

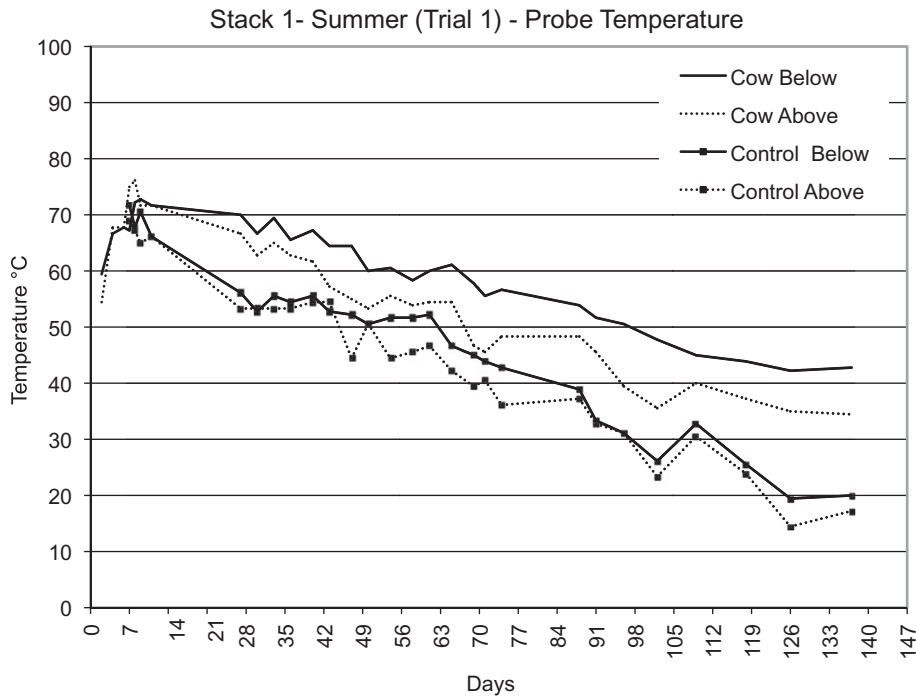


Figure 3. Trial 2, Winter: Probe temperatures (°C) manually taken above and below, left and right, carcasses by probing the pile with a 3-foot long temperature probe during the composting period.

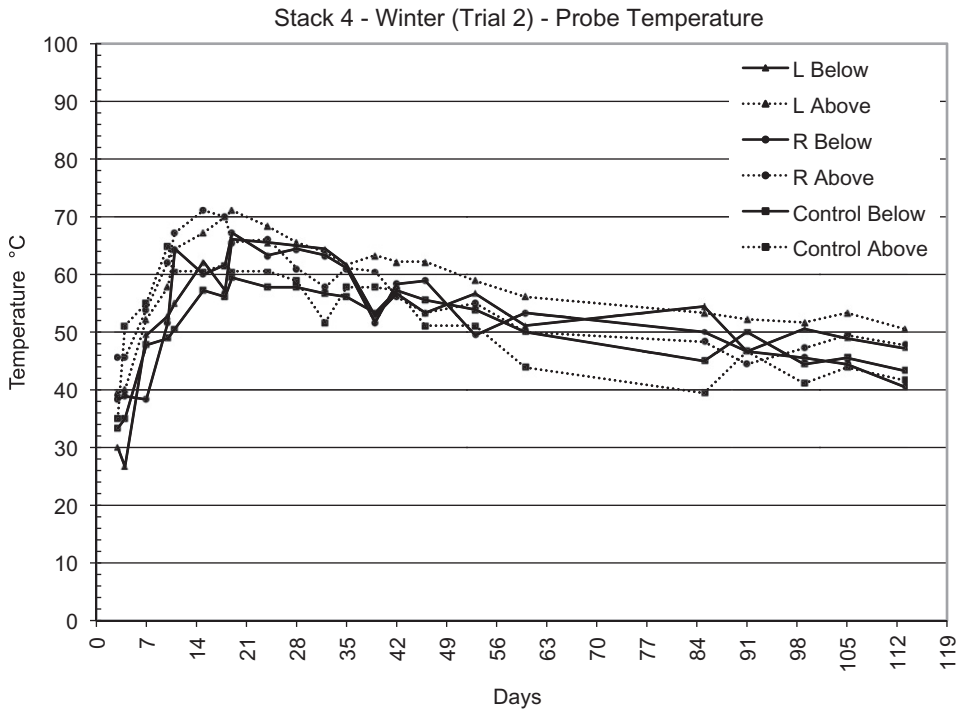


Figure 4. Monthly total precipitation in the Visalia area during both composting trial periods during 2008 and 2009.

removed from the composting stacks and taken to the CAHFS laboratory for disposal. Bones were primarily what remained of the carcasses, along with a few pieces of hide and charred muscle fragments (Figure 5). The carcass parts other than bones that remained were found at the bottom of the piles next

to the soil. The bones remaining from two carcasses fitted into a single 0.2m³ barrel. The remaining composting material was reduced from approximately 80 m³ to approximately 20 m³ (four truck loads to approximately one truck load). Material remaining after the winter trial was similar to the summer trial,

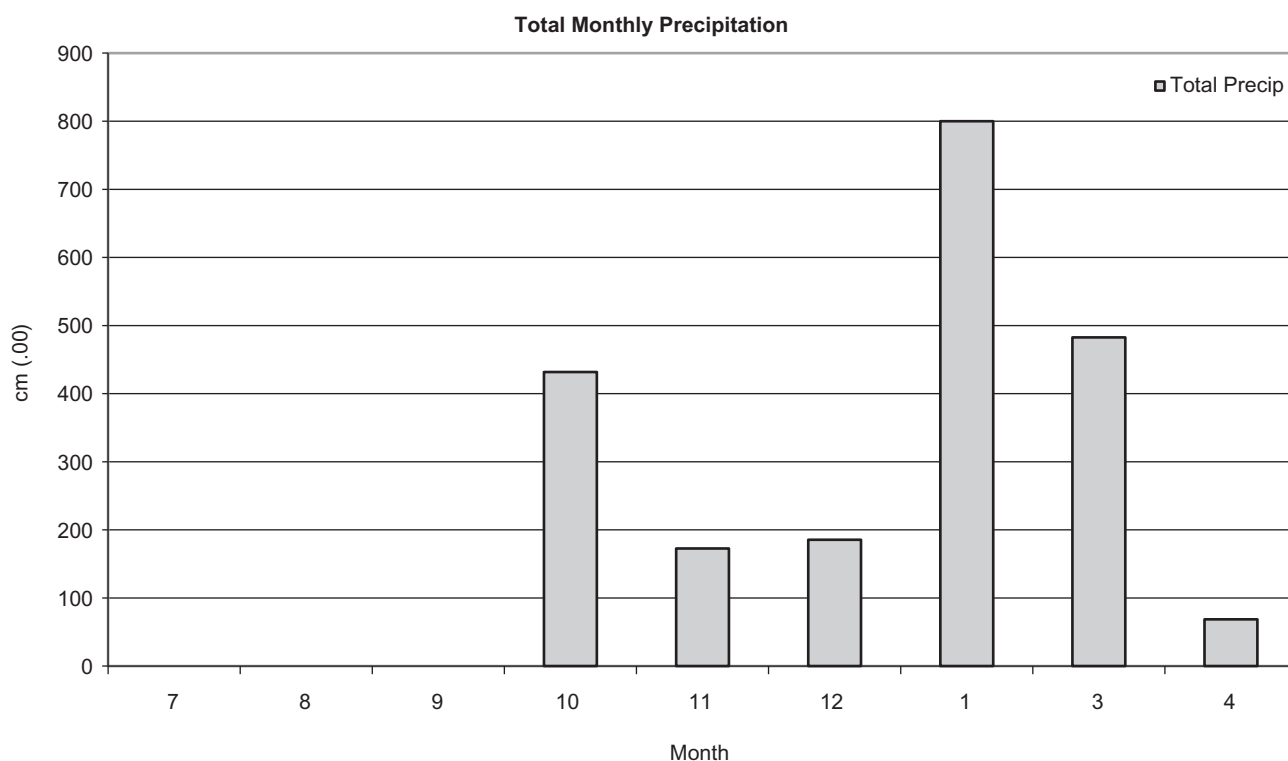


Figure 5. During the winter trial (Trial 2), there were places within the carcasses that were not completely composted by 128 to 143 days, as evidenced by small amounts of tissue still connected to the bones. Some of these areas are shown in this picture.

although there were more areas of large muscle mass that were not completely composted. This was noted in both sets of stacks regardless of the number of composting days. The incompletely composted areas were primarily found on the lower portions of the carcasses, where the composting materials had been compacted.

Odours, insects and wildlife

During the composting period for both trials, the predominant odour from the piles was that of the recycled manure used as the composting material. No odour of decomposing flesh was noted by the staff collecting pipe samples from under the piles. Flies were not attracted to the piles. After the crust formed on the surface during Trial 2, large numbers of ants began to colonise the piles. Despite the rodent control programme, local ground squirrels were seen occasionally on the piles and one squirrel burrow was noted under one of the soil platforms. No signs of attempts by wildlife to dig out the carcasses were seen during the composting period.

Soil composition

In both trials, more leachate appears to have penetrated to the bottom row of pipes within the platforms

filled with sandy soil compared to those filled with clay soil, although data is limited. This is based on the numbers of pipe samples with bacteria count $>1 \times 10^6$ CFU/ml on the sandy soil (48%) and clay soil (30%).

Discussion

Proper composting procedures for large animal carcasses require managing the variables of oxygen content, moisture level, carbon to nitrogen ratio and amount of 'co-composting' material. If properly managed, composting will dispose of large animal mortalities and can achieve degradation of carcasses in as little as 120 days with temperatures high enough to effectively inactivate pathogens (Mukhtar et al. 2004). The final product is compost-like material that can be reused as an amendment to compost more carcasses. Material from fully composted mortalities can be reused as a mortality composting amendment provided it does not exceed 50% of the total amendment (Glanville et al. 2006). Composting small carcasses, such as poultry, creates a uniform pile where regular mixing can occur to maintain proper oxygenation. Successful decomposition of small carcasses in a uniform pile can be achieved in as little as 60 days (Gonzalez & Sanchez 2005). The size of carcasses from large animals prevents a compost pile from being uniform. Regular mixing of the pile is not practical in the early stages of

composting when carcasses are largely intact and decomposition is still progressing. Large animal composting requires longer periods to achieve successful decomposition. Kalbasi et al. (2006) describes design, capacity, and by-products from large mortality composting. If proper attention is given to the design and layout, and the pile is properly monitored, the environmental impact can be managed and the final composted material can be an acceptable product (Kalbasi et al. 2006). In our study, cows were composted in contained bins, with 45 cm of top and bottom cover material consisting of SDMS manure. After 130 days, composting achieved an almost complete loss of all flesh and muscle.

Rate of decomposition

In our study, SDMS (screened dairy manure solids) was used as the composting amendment for carcass decomposition. Although the moisture level of the SDMS we used was not measured, typical levels for SDMS range from 70 to 80% moisture, similar to that of silage. In California, manure that is routinely scrapped from corral surfaces (dry lot scrapings), or SDMS that has been sun-dried for use as free stall bedding is much drier, typically ranging from 15 to 20% moisture. This distinction is important, since many might consider dry lot scrapings and sun-dried, screened manure solids as composted manure, as its gross physical appearance can be similar to properly composted manure. Proper composting, however, requires sufficient moisture and microbial activity to raise temperatures to inactivate pathogens. SDMS was chosen as the composting amendment for our study, because in California, dairies are large (925 cows/herd average) and SDMS is readily available in sufficient quantities to support mortality composting, even if a need arose for additional carcass disposal capacity in the event of an emergency declaration. Dry lot scrapings, which are also readily available, were not investigated in this trial.

In this study, carcasses were reduced primarily to bones in 130–140 days. In a study in Iowa, Glanville (2006) achieved complete decomposition 4 to 6 months in warm weather and 8 to 10 months in winter weather with silage, cornstalks, and straw/manure as co-compost amendments. During the winter in Iowa, January temperatures on average are 13 to 17° lower ($-3.89^{\circ}\text{C}/-12.78^{\circ}\text{C}$ Hi/Low) than California ($8.88^{\circ}\text{C}/3.89^{\circ}\text{C}$ Hi/Low). Longer composting periods with some turning to introduce more oxygen into the pile could be expected to provide more complete composting. In our study, the tissue remaining after

composting for 130 days lacked any definitive muscular characteristics, and the lack of complete breakdown was most likely related to the evaporation of moisture below optimal levels in California's dry climate. This was evidenced by spikes in pile temperatures following rain events. There was compaction in the lower areas of the piles, which was probably due to the weight of the carcasses and composting materials around and above the carcasses. In Trial 2, the unopened carcasses (carcasses in stacks 5 and 6) had similar, incompletely composted areas in the lower portions of the composting piles. The composting piles were also reduced to approximately one-fourth of their original volume.

Pile temperatures

The generation of heat by bacteria that is high enough to inactivate pathogens during proper composting requires the pile to be sufficiently oxygenated to create an aerobic environment for decomposition. Without turning the piles, as is the case for the primary stage of composting large carcasses, proper oxygenation can be achieved with the amendments used, provided they have sufficient porosity and mechanical strength to prevent compaction and allow gas exchange (Glanville et al. 2006). Oxygen levels of 10% to 15% were achieved in the inner core of large carcass compost piles with several materials, including ground corn stalks, silage and a straw manure mixture. Oxygen levels were much higher at the carcass surface and outer core with levels of 15% to 18% oxygen (Glanville et al. 2006). Our study was designed to approximate the most likely and most practical farm application of large carcass composting for California conditions, using readily available SDMS as the composting amendment. After placement of the carcasses, the only manipulation during the 120–137 day observation period was to add an additional 30 cm of SDMS half-way through the composting period. For all piles and seasons, the pile temperatures were above 55 °C for at least 21 days. The continuous monitoring temperature probes matched manual probing of the pile for temperature readings, with the exception of stack 4 during the winter season, which did not achieve 55 °C with automated recording devices, although it did with a temperature probe.

The Environmental Protection Agency has classified bio-solids (composted municipal sewage sludge) into two categories. Class A bio-solids must have pathogens reduced to very low levels to the extent that restrictions on end use and handling are minimal. Class B bio-solids have a less rigorous pathogen

reduction standard, so prescribed measures and restrictions are required for utilisation (e.g. time period before grazing after land application) (USEPA 1992). Temperatures for Class A bio-solids need to achieve a temperature of at least 55 °C/131 °F for at least 3 days and Class B is 40 °C/104 °F for 5 days with 4 hours at 55 °C. The ideal temperature range to kill pathogens but not damage microorganisms that are beneficial to the composting process is between 55 °C and 65 °C. The temperatures within the piles for our study maintained >60 °C for more than 20 days, exceeding Class A and B bio-solids time/temperature requirements by 5 to 10-fold. Despite physical and chemical differences in municipal sludge and animal wastes, knowledge of the microbial standards for sludge provides useful guidelines for carcass composting (Sander et al. 2002).

Pathogen inactivation in the primary and secondary phase of composting can be reduced if there is clumping of solids, non-uniform temperatures below required levels, or if pathogens are reintroduced at the end of the heating phase (Haug 1993). Avoiding these unfavourable conditions for proper composting can be achieved with uniform airflow and temperatures throughout the composting process (Keener et al. 2000). High core temperatures can ensure that proper composting is taking place and is acting as a pasteurisation process. Pathogenic bacteria levels are reduced when the middle of the pile reaches 65 °C in two days (Glanville & Trampel 1997). Internal pile temperatures in composting swine mortalities in the thermophilic range (for composting, beneficial thermophilic bacteria grow at temperatures over 100 °C) for extended periods of one to several weeks was reported to be sufficient to inactivate the *Pseudorabies* virus, *Salmonella* spp., and *Actinobacter pneumonia* (Harper et al. 2001). In our study, pile temperatures were sustained above Class A and Class B bio-solid levels for extended periods, not only within the carcasses and in the areas above and below the carcasses, but also in the control sections of the pile. This was probably due to adequate moisture and oxygen levels for microbial activity in the SDMS used as the co-composting amendment.

Carcass leachate and environmental effects

In our study, it appears that the majority of the liquids from the carcasses, approximately 0.36 m³/540 kg carcass (Smith 1996), were absorbed into the compost materials surrounding the carcasses. All the water from the carcasses or rainfall was contained within the composting platforms above the plastic liners. Rainfall early in the composting period (less than 60 days) initiated a meaningful increase in compost temperature, which

aided carcass decomposition. However, temperature rises later in the composting period (after 90 days) were inconsequential, as the stack temperatures were mostly below 38 °C at this time. No problems with odour, flies or wildlife were noted during the composting period.

Bacterial levels

In a study of static compost piles of dairy manure, separator solids were shown to have coliform bacteria levels greater than 1 × 10⁸ CFU/gm at the beginning of the composting period. During the composting period, there was a reduction of up to 4 logs in coliform bacterial levels after 20 days, with a mean maximum temperature of 65 °C (Mote et al. 1988). In this study on static piles, coliform levels frequently returned to pre-composting levels at 80 days, the end of the study, indicating that regrowth had occurred. This contrasted to a composting study of feedlot manure and bedding, where frequent turning of the compost pile occurred and after 7 days, 6 logs of bacteria were eliminated and regrowth of coliforms did not occur (Larney et al. 2003). In our study, the leachate in the soil layer was sampled for total bacteria, and in Trial 1, 75 of 380 pipe samples (19.7%) exceeded 1 × 10⁶ CFU per ml of the total bacterial count for carcasses composted with recycled manure. In Trial 2, 50 of 262 samples (19.1%) exceeded this count. In Trial 1, 40 of these samples were found in swabs from the pipes at the 20 cm level, compared to 35 for pipes at the 45 cm level. Eleven of the pipes had repeated, consecutive counts greater than those found in normal, recycled manure solids. Almost 70% of the samples that exceeded the expected levels for recycled manure were found in swabs taken on Day 7 of the study. In Trial 2, 36 samples >1 × 10⁶ were from the upper pipes and 14 from the lower pipes. Similar numbers of isolates were found at each sampling date in Trial 2. The most numerous isolates in both trials were Gram-negative bacteria.

Conclusions

The results provided by statistical analysis allows us to draw some conclusions. As regards the summer it can be stated that there is a clear relationship between the ambient temperature and the temperature inside the piles as a whole and that the duration of the composting process is not affected by air temperature. However, analysing the relationship between the two variables above with each single stack it can be noted that in two out of four stacks there is a more significant relationship between the dependent variable

(internal temperature) and one of the independent variable (external temperature), while in the remaining two stacks it is preponderant in the significance the role of another variable that is the time elapsed although both cooperate to improve the model performance.

As regards instead the winter season, the independent variables are the Ambient temperature, the Time elapsed and the Precipitations. The analysis shows how the three independent variables were acting synergistically on the internal temperature. In winter, rainfall is very abundant in the early part of the season and this surely has caused the cold atmospheric water reached even the core of the pile, lowering his own temperature. Furthermore, the clay coverage, given the high microporosity of the micelles, with strong precipitations is both imbibed and thanks to the low outside temperature has meant that for a certain period of time the 'environmental' conditions are not the most favourable for the proliferation of microbial mass.

Despite these limited composting trials, the results indicate, in the climate context of California, that composting can be expected to reduce large livestock carcasses to bones without significant impact on the nearby environment. Composting can also be anticipated to provide temperatures capable of destroying most pathogens within the carcasses. The distributions of the frequencies of bacterial loads are independent in summer from winter and vice versa. Based on these results, the managerial and policy implications are that composting should be considered as a safe, alternative method of carcass disposal, when rendering capacity is overwhelmed or unavailable (Eggerth et al. 2007).

Acknowledgements

This work was funded by the Centre for Food Animal Health, School of Veterinary Medicine, University of California, Davis. Thanks to the faculty members of the California Animal Health & Food Safety Laboratory at the VMTRC, the California Department of Food and Agriculture, Veterinary Medical Officers at the Tulare, Animal Health Branch, and Christina VanWorth, Stephanie Jimenez, Erin Sing of the Dairy Food Safety Laboratory, VMTRC for their assistance in this study. The Authors greatly valued Prof. L. C. Piccinini comments and suggestions and express sincere appreciation to members of Polyglot, Perugia, for a careful reading and comments on the manuscript.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was funded by the Centre for Food Animal Health, School of Veterinary Medicine, University of California, Davis. Thanks to the faculty members of the California Animal Health & Food Safety Laboratory at the VMTRC, the California Department of Food and Agriculture, Veterinary Medical Officers at the Tulare, Animal Health Branch, and Christina VanWorth, Stephanie Jimenez, Erin Sing of the Dairy Food Safety Laboratory, VMTRC for their assistance in this study

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