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REDUCTION OF FOODBORNE PATHOGENS DURING CATTLE MANURE COMPOSTING WITH ADDITION OF CALCIUM CYANAMIDE

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Abstract. Inactivation of four species of foodborne pathogens (*E. coli*, *Salmonella*, *E. faecalis*, and *S. aureus*) was investigated during laboratory-scale composting of fresh cow manure with addition of calcium cyanamide ($CaCN_2$) at constant temperature conditions, and the effects of temperature and additive content of $CaCN_2$ on the efficacy of inactivation were evaluated. At different composting temperatures (20, 30, 37 and 50 °C), a significant inhibition or quick inactivation of pathogens were observed during 10 d composting with addition of 2.0% $CaCN_2$, and the effect was more obvious at mesophilic temperatures compared to thermophilic temperature. Therefore, the ideal additive content of $CaCN_2$ was determined at 30 °C through mixing 2.0%, 2.5%, and 3.0% $CaCN_2$ with manure. With increase in additive content of $CaCN_2$, the efficacies of pathogen inactivation also increased. However, the result indicates that, no less than 2.5% $CaCN_2$ should be mixed with the manure to entirely eliminate the pathogens during composting.

Keywords: foodborne pathogen; calcium cyanamide; cow manure; composting; waste management technologies.

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Introduction

Manure of livestock and poultry carry some of the important environmental pollutants like organic materials, sediments and microorganisms. In this case, the contamination from pathogens in animal manure is becoming more and more dominant and serious with the explosive development of animal husbandry industry and with the increase in environmental protection awareness. Several livestock species and poultry carry large populations of foodborne pathogens, such as Escherichia, Salmonella and Campylobacters in their manure (Doyle, Erickson 2006; Viazis, Diez-Gonzalez 2011). These pathogens originate from the animal's intestinal tract and typically shed in the tract asymptomatically (Doyle, Erickson 2006). Pathogens carried in manure can be easily transmitted by other vectors from manure to animals, produce, or humans (You *et al.* 2006; Martens, Böhm 2009). Therefore, the factors in this transmission pathway need be intervened to prevent the fecal pathogen's contaminations or infections. And in these interventions, sanitation of manure is considered as an effective way to control the contaminations on site.

Composting is not only the most efficient process to produce an agronomically advantageous soil organic amendment, but also one of the most environmentally friendly treatments to inactivate pathogenic organisms or reduce them to an acceptable levels (Fernández *et al.* 2007; Wichuk, McCartney 2007). Up to now, much work has been done by the researchers to investigate the pathogen destructive and disease suppressive effect of composting worldwide. Temperature plays an important role in the fate of pathogens. The efficiency of pathogen inactivation depends not only on the degree of temperature, but also on the exposure time of pathogens to heat. However, some foodborne pathogens have a great capacity to survive for long periods in manure. In a laboratory-scale bioreactor, *E. coli* O157:H7 still survived in manure composting at 21 °C after 36 d, while not detected 14 d post-composting at 50 °C

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(Jiang *et al.* 2003). *Salmonella* serovar Newport even surprisingly persisted for a long time, 184 d in dairy cow manure at ambient temperature (24.5 ± 1.4 °C) in laboratory (You *et al.* 2006). And *Salmonella typhimurium* was culturable for up to 28 d and 42 d in swine manure in thermophilic composting at 55 °C and aerated liquid storage, respectively (Grewal *et al.* 2007). These results demonstrated that even under thermal conditions, some strains of pathogens could exist for a long time in manure. Such great persistence increases the chances of an outbreak occurring and further increases the environmental load. Therefore, it is necessary to produce more reliable and realistic methods feasible in both sanitation and recycling of manure.

Calcium cyanamide ($CaCN_2$) is not only a good fertilizer. It has also shown good performance in the suppression of soilborne pathogens. $CaCN_2$ effectively suppressed *Fusarium solani* f.sp. *cucurbitae* in greenhouse cucumber (Bourbos *et al.* 1997), and was also fungicidal to *Fusarium oxysporum* f. sp. *Cucumberinum* (Shi *et al.* 2009). However, very little is known about its effect on zoonotic microorganisms. Our research thus studied the impact of $CaCN_2$ on foodborne pathogens from dairy cow manure origins during composting, attempting to remedy the defect about pathogen inactivation in manure treatment.

1. Materials and methods

1.1. Raw composting materials

Fresh manure containing 87.3% moisture content, 37.3% carbon and 1.6% nitrogen was obtained from healthy dairy cows at a local dairy farm. The manure was sent to laboratory immediately after collection in portable ice boxes and processed for experiment on the same day. $CaCN_2$ was an imported product of Guangzhou WeiBo Chemical Co., Ltd., China, containing 21.3% N and 58% CaO, and granule size was 0.2–2 mm diameters.

1.2. Composting temperature

In the experimental group, $CaCN_2$ was thoroughly mixed with 1200 g fresh manure at an additive content of 2.0% (weight percentage), and then four equal portions of mixtures (each 200 g) were respectively transferred into a 400 ml glass bottle and composted at different constant temperatures for 10 d in thermostatic incubators (JC–SPJ–480, Jinan Jingcheng Experimental Instrument Co., Ltd., China). The control group was with no $CaCN_2$ mixed and ran the same procedures as the experimental group. In details, the experimental design used was a 2-factor factorial design of four temperatures (T) × two additive contents (D) (Liang *et al.* 2003). The four temperature settings were 20, 30, 37 and 50 °C, and the two additive contents were 0% and 2.0%. Every treatment was at one temperature setting with two additive contents settings.

1.3. Additive content of $CaCN_2$

In the experimental group, $CaCN_2$ was thoroughly mixed with 200 g fresh manure at the additive contents of 2.0%, 2.5%, and 3.0% in 400 ml glass bottles, respectively. And the mixtures were composted for 5 d at a constant temperature of 30 °C, which was selected as a representative composting temperature for $CaCN_2$ inhibition against pathogens. The control group was with no $CaCN_2$ mixed and ran the same procedures as the experimental group.

1.4. Sampling and pathogen enumeration

For sampling, 1.0 g of composting sample in duplicate was collected from each treatment at 0, 1, 3, 5, 7 and 10 d in the determination of a suitable composting temperature and at 0, 1, 2, 3 and 5 d in the determination of a suitable additive content. Then, the samples were analyzed for the changes of pathogen population during composting. The tested pathogens were *E. coli*, *Salmonella*, *Enterococcus faecalis* (*E. faecalis*), and *Staphylococcus aureus* (*S. aureus*).

For pathogen enumeration, serial dilutions (1:10) of composting samples with sterile physiological saline were performed and homogenized for 10min in a rotatory stirrer. 0.1 ml aliquots of each dilution were spread onto the appropriate agar plates in triplicate. All agar plates were incubated at 37 °C for 24 h, and colonies were counted. Thirty to three hundred colonies per plate were considered to be optimal countable numbers. *E. coli* was investigated from eosin-methylene blue agar (EMB) plates and confirmed by Indole Test. XLD (xylose-lysine-deoxycholate) agar was used for detection of *Salmonella*, while KF streptococci agar and Baird-Parker agar were for *E. faecalis* and *S. aureus*, respectively. When the concentration of pathogen dropped below the quantitative detection limit (100 CFU/g) of direct plating method, selective enrichment was used to assay for the presence of pathogens. The used enrichment broths were Luria-Bertani (LB) broth for *E. coli*, Rappaport-Vassiliadis (RV) enrichment broth and Selenite cystine (SC) enrichment broth for *salmonella*, Azide glucose (AD) broth for *E. faecalis*, and Soy bean casein digest broth with lecithin and polysorbate medium (SCDLP) and Manitol salt (MS) broth for *S. aureus.* After incubation for 24 h at 37 °C, the enrichment cultures were inoculated onto the surface of the selective agar plates as mentioned above and streaked for isolated colonies.

1.5. Statistical analysis

Data were analyzed using ANOVA for variances and Duncan's procedure from SAS (version 9.0) for multiple comparisons.

2. Results

2.1. Influence of composting temperature on $CaCN_2$ inhibition against foodborne pathogen

The populations of four pathogens in initial manure and the changes during composting at different temperatures are shown in Tables 1 and 2.

In the experimental group (Table 1), *E. coli* was never detected from 5, 7 and 7 d at 20, 30 and 37 °C, respectively. But, *Salmonella*, *E. faecalis*, and *S. aureus* were still detected until the end of experiment. At these three temperatures, the populations of three microorganisms respectively decreased 59.28, 62.30 and 41.58% for *salmonella*, 28.71, 44.12 and 57.80% for *E. faecalis*, and 30.72, 55.88, and 39.18% for *S. aureus* at the end of composting compared with the initial values. At 50 °C, no growth of *E. coli* and *salmonella* was observed from 1 d, while that of *E. faecalis* was from 3 d. However, *S. aureus* was thoroughly undetectable by enrichment culture just at 10 d when it did not decrease the detection limit of direct plating until 7 d.

In the control group (Table 2), the survival of all four pathogens was observed at the end of experiment at 20, 30 and 37 °C. At these three temperatures, the populations of *E. coli* respectively decreased 6.58, 9.30 and 4.01% at the end of composting compared with the initial values. For *salmonella*, the corresponding decreases were 33.48, 30.17 and 27.30%, while that were 37.17, 39.17 and 50.00% for *E. faecalis*, and 24.43, 17.81 and 16.56% for *S. aureus*. At 50 °C, *E. coli*, *Salmonella*, and *E. faecalis* were thoroughly undetectable from 3 d except *S. aureus* was still observed at the end of composting. Among them, *E. coli* and *Salmonella* were not detected from 1 d and *E. faecalis* was from 3 d.

Log removal for four pathogens during composting at different temperatures was calculated. At 50 °C, *E. coli* and *salmonella* either in the experimental group or in the control group was all not observed from 1 d. So, their log removals should be in the highest levels, and to exhibit this law and calculate average log removal, pathogen concentration (log CFU/g) in the sampling days in which pathogen was not detected or under the detection limit was taken a value of zero. In the experimental group, an average log removal for *E. coli* was respectively 4.72, 3.62, 3.95 and 5.74 at 20, 30, 37 and 50 °C, when correspondingly 3.29, 3.35, 3.18 and 6.95 for *Salmonella*, 1.52, 1.55, 1.86 and 4.39 for *E. faecalis*, and 0.90, 1.45, 1.27 and 2.94 for *S. aureus*. In the control group, the average log removal was −0.33, −0.12, 0.24 and 6.99 for *E. coli* at 20, 30, 37 and 50 °C, respectively, which was 1.56, 1.26, 1.36 and 6.96 for *Salmonella*, 1.48, 1.27, 1.79 and 4.89 for *E. faecalis*, and 0.58, 0.38, 0.17 and 0.95 for *S. aureus*.

Summing up, an average log removal for four pathogens was in the range of 0.90–4.72 at 20 °C, 1.45–3.62 at 30 °C, 1.27–3.95 at 37 °C and 2.94–6.95 at 50 °C in the experimental group, while it was correspondingly −0.33 to 1.56, −0.12 to 1.27, 0.17 to 1.79, and 0.95 to 6.99 in the control group. As to a general average log removal for all pathogens at different temperatures, it was respectively 2.61, 2.49, 2.57 and 5.00 at 20, 30, 37 and 50 °C in the experimental group, and was 0.82, 0.70, 0.89 and 4.95 in the control group.

2.2. Influence of $CaCN_2$ on foodborne pathogen during mesophilic composting

Among the mesophilic temperatures (20 °C, 30 °C, and 37 °C), 30 °C was selected to determine an ideal additive content of $CaCN_2$ in composting against the tested pathogens. The selection of the temperature was based on the results of experiment 3.1. and the consideration of initial temperature degree of composting process. As shown in Table 3, the treatments mixed with 2.5% and 3.0% $CaCN_2$ revealed good inhibitory effects on the growth of four microorganisms. And with increase inadditive content, the inhibitory efficacy was correspondingly improved. In the treatments mixed with 3.0% $CaCN_2$, the survival of *E. coli* was not observed from 1 d, when that of *Salmonella*, *E. faecalis*, and *S. aureus* was from 2 d. In the treatments mixed with 2.5% $CaCN_2$, *E. coli* was not detected from 2 d; *Salmonella*, *E. faecalis*, and *S. aureus* were all from 3 d. By contrast, a long persistence of four microorganisms appeared in the treatments mixed with 2.0% $CaCN_2$ and in the control group, that the survival of all microorganisms was still observed at the end of the experiment. The population of *E. coli* decreased below the detection limit of direct plating at 5 d in the treatments mixed with 2.0% $CaCN_2$. But in the control group, it was always detected in large quantities during the entire time of composting, decreasing only 6.36% at the end of composting in comparison to the initial value. *Salmonella*, *E. faecalis*, and *S. aureus* were all detected in direct plating in the two groups. At the end of the composting, the populations of *Salmonella* in comparison to the initial value decreased 67.15% and 22.63% in the treatments mixed with 2.0% $CaCN_2$ and control group, respectively, while the values were 42.18% and 35.68% for *E. faecalis*, and 57.40% and 19.27% for *S. aureus*.

Log removal for four pathogens during composting with addition of different contents of $CaCN_2$ at 30 °C is presented in Table 4. In the experimental group, an average log removal for *E. coli* was rescpectively 2.97, 5.34 and 6.45 in the treatments

Table 1. The survival and persistence of *E. coli*, *Salmonella*, *E. faecalis*, and *S. aureus* during cattle manure composting with addition of 2.0% $CaCN_2$ at different temperatures

Microorganism	Composting temperature (°C)	Mean[a] ± SD (log CFU/g) at sampling time (d)					
		0	1	3	5	7	10
E. coli	20	5.74 ± 0.07A[b]a[c]	5.08 ± 0.02Cb	<2.0[d]	ND	ND	ND
	30	5.74 ± 0.07Ab	5.91 ± 0.11Aa	4.68 ± 0.02c	<2.0	ND	ND
	37	5.74 ± 0.07Aa	5.38 ± 0.04Bb	<2.0	3.57 ± 0.16c	ND	ND
	50	5.74 ± 0.07A	ND	ND	ND	ND	ND
Salmonella	20	6.95 ± 0.18Aa	4.35 ± 0.24Db	4.09 ± 0.16Ab	3.90 ± 0.16Ab	3.11 ± 0.15Ab	2.83 ± 0.35Ab
	30	6.95 ± 0.18Aa	4.62 ± 0.31Bb	3.96 ± 0.18Bb	3.86 ± 0.12Bb	2.96 ± 0.20Bb	2.62 ± 0.16Bc
	37	6.95 ± 0.18Aa	5.53 ± 0.17Cb	2.72 ± 0.15Db	2.57 ± 0.14Db	3.97 ± 0.14Da	4.06 ± 0.19Ca
	50	6.95 ± 0.18A	ND	ND	ND	ND	ND
E. faecalis	20	5.19 ± 0.03Aa	4.14 ± 0.33Ab	3.68 ± 0.05Cc	3.39 ± 0.06Bd	3.44 ± 0.04Acd	3.70 ± 0.16Ac
	30	5.19 ± 0.03Aa	4.08 ± 0.11Ab	4.25 ± 0.04Ab	3.69 ± 0.07Ac	3.29 ± 0.05Bd	2.90 ± 0.14Ac
	37	5.19 ± 0.03Aa	4.11 ± 0.08Ab	3.97 ± 0.08Bb	3.68 ± 0.27Ac	2.70 ± 0.05Cd	2.19 ± 0.10Ac
	50	5.19 ± 0.03Aa	4.01 ± 0.03Ab	ND	ND	ND	ND
S. aureus	20	4.85 ± 0.01Aa	4.70 ± 0.03Cb	4.21 ± 0.04Ac	3.99 ± 0.07Ad	3.51 ± 0.02Ae	3.36 ± 0.11Af
	30	4.85 ± 0.01Aa	4.18 ± 0.07Bb	3.74 ± 0.02Cd	3.81 ± 0.03Bc	3.12 ± 0.04Ce	2.14 ± 0.00Cf
	37	4.85 ± 0.01Aa	4.13 ± 0.02Db	3.89 ± 0.05Bc	3.64 ± 0.02Cd	3.28 ± 0.08Be	2.95 ± 0.03Bf
	50	4.85 ± 0.01Aa	3.89 ± 0.03Bb	3.56 ± 0.02Dc	2.11 ± 0.05Dd	<2.0	ND

ND, not detected after enrichment.
[a]The mean is the average of six replicates.
[b]For each pathogen, means with different upper case letters in a column are significantly different ($P < 0.05$).
[c]Means with different lower case letters in a row are significantly different ($P < 0.05$).
[d] <2.0 log CFU/g, positive after enrichment.

Table 2. The survival and persistence of *E. coli*, *Salmonella*, *E. faecalis*, and *S. aureus* during cattle manure composting with no $CaCN_2$ mixed at different temperatures

Microorganism	Composting temperature (°C)	Mean[a] ± SD (log CFU/g) at sampling time (d)					
		0	1	3	5	7	10
E. coli	20	6.99 ± 0.04A[b]d[c]	6.66 ± 0.03Ce	8.32 ± 0.02Aa	7.88 ± 0.08Ab	7.23 ± 0.01Bc	6.53 ± 0.16Bf
	30	6.99 ± 0.04Ae	7.53 ± 0.11Ba	7.11 ± 0.06Bd	7.40 ± 0.13Bb	7.15 ± 0.09Cc	6.34 ± 0.02Cf
	37	6.99 ± 0.04Ab	7.58 ± 0.16Aa	6.83 ± 0.01Cc	5.08 ± 0.17Ce	7.56 ± 0.11Aa	6.71 ± 0.01Ad
	50	6.99 ± 0.04A	ND	ND	ND	ND	ND
Salmonella	20	6.96 ± 0.13Ad	6.95 ± 0.23Bd	5.86 ± 0.17Aa	4.91 ± 0.12Ac	4.65 ± 0.20Ab	4.63 ± 0.26Ae
	30	6.96 ± 0.13Aa	6.94 ± 0.22Cb	5.93 ± 0.26Cc	5.80 ± 0.21Cd	4.96 ± 0.35Be	4.86 ± 0.31BCf
	37	6.96 ± 0.13Ab	7.99 ± 0.21Aa	5.04 ± 0.21Dc	5.45 ± 0.23Dc	4.46 ± 0.09Bc	5.06 ± 0.23BCc
	50	6.96 ± 0.13Aa	ND	ND	ND	ND	ND
E. faecalis	20	6.00 ± 0.07Aa	5.80 ± 0.23Aa	4.89 ± 0.15Bb	4.22 ± 0.23Bb	3.94 ± 0.04Bc	3.77 ± 0.06Ad
	30	6.00 ± 0.07Aab	6.15 ± 0.11Aa	4.96 ± 0.08Ab	4.66 ± 0.06Ac	4.23 ± 0.15Ad	3.65 ± 0.06Be
	37	6.00 ± 0.07Aa	6.03 ± 0.14Aa	4.71 ± 0.08Bb	3.74 ± 0.15Cc	3.58 ± 0.06Bc	3.00 ± 0.10Bd
	50	6.00 ± 0.07Aa	5.55 ± 0.07Bb	ND	ND	ND	ND
S. aureus	20	4.83 ± 0.02Aa	4.74 ± 0.07Cc	4.77 ± 0.06Cb	4.25 ± 0.05Dd	3.84 ± 0.04De	3.65 ± 0.01Cf
	30	4.83 ± 0.02Ab	4.80 ± 0.05Bc	4.91 ± 0.03Ba	4.44 ± 0.01Bd	4.12 ± 0.08Be	3.97 ± 0.04Bf
	37	4.83 ± 0.02Ac	5.24 ± 0.01Ab	5.28 ± 0.03Aa	4.53 ± 0.05Ad	4.21 ± 0.02Ae	4.03 ± 0.06Af
	50	4.83 ± 0.02Aa	4.72 ± 0.02Db	4.24 ± 0.01Dd	4.38 ± 0.05Cc	3.86 ± 0.03Ce	2.21 ± 0.07Df

ND, not detected after enrichment.
[a]The mean is the average of six replicates.
[b]For each pathogen, means with different upper case letters in a column are significantly different ($P < 0.05$).
[c]Means with different lower case letters in a row are significantly different ($P < 0.05$).

mixed with 2.0%, 2.5% and 3.0% $CaCN_2$, which was 3.21, 5.11 and 6.76 for *Salmonella*, 1.38, 4.57 and 4.80 for *E. faecalis*, and 1.58, 4.05 and 4.17 for *S. aureus*. In the control group, the average log removal was −0.12, 0.78, 0.78 and 0.71 for *E. coli*, *Salmonella*, *E. faecalis* and *S. aureus*, respectively. Generally, an average log removal for four pathogens was in the range of 1.38–3.21, 4.05–5.34 and 4.17–6.76 in the experimental treatments mixed with 2.0%, 2.5% and 3.0% $CaCN_2$, while it was only −0.12 to 0.78 in the control group. A general average log removal for all pathogens was 2.29, 4.77 and 5.54 in 2.0%, 2.5% and 3.0% $CaCN_2$ treatment when 0.54 in the control group.

3. Discussion

Thermal treatment is one of the most efficient ways to inactivate pathogens during composting. However, it has been found that some foodborne pathogens could persist for long periods in manure even under thermal composting conditions (Jiang *et al.* 2003; Grewal *et al.* 2007). The result of the present study also confirmed the observations. During composting of cow manure at 50 °C for 10 d, the survival of *S. aureus* was still observed at the end of composting with the population of 2.21 log CFU/g. Nevertheless, under the same conditions, *E. coli* and *Salmonella* were not detected from 1 d; *E. faecalis* was undetectable from 3 d. So, the results of our study and other observations indicate that survival of different pathogens in manure during composting differ from each other in some extent. For this reason, the guarantee of complete elimination of different pathogens, especially the human and animal infectious pathogens, in compost previously to its application has become a tough task to be solved immediately with the increase of the cases of foodborne diseases and the emergence of new foodborne pathogens.

$CaCN_2$ has been mainly used as a fertilizer for a long time (Oh *et al.* 2006; Takeda *et al.* 2007; Soltys *et al.* 2011). However, in recent years, several studies reported its fungicidal effect on the pathogens of the soilborne diseases (Bourbos *et al.* 1997; Shi *et al.* 2009). With consideration of both advantages and to evaluate the role of $CaCN_2$ as an antimicrobial agent during composting, we studied its effect on foodborne pathogens during cow manure composting in the present study. And the results confirmed its inhibitory effect on these microorganisms. With addition of $CaCN_2$, *E. coli*, *Salmonella*, *E. faecalis*, and *S. aureus* in manure were inactivated or reduced significantly quicker than that in the treatments with no $CaCN_2$ mixed during composting. Meanwhile, the inhibitory efficacy of $CaCN_2$ against the microorganisms was influenced by composting temperature and additive content of $CaCN_2$. The inactivation at 50 °C was more rapid and effective than at 20 °C, 30 °C, and 37 °C. The result was the same both for the experimental group and control group. Therefore, the phenomenon was

Table 3. The survival and persistence of *E. coli*, *Salmonella*, *E. faecalis* and *S. aureus* during cattle manure composting with addition of different contents of $CaCN_2$ at 30 °C

Microorganism	Additive content[a] (%)	Mean[b] ± SD (log CFU/g) at sampling time (d)				
		0	1	2	3	5
E. coli	0[c]	6.45 ± 0.06A[d]d[e]	6.90 ± 0.07Aa	6.76 ± 0.11Ab	6.57 ± 0.21Ac	6.04 ± 0.11e
	2.0	6.45 ± 0.06Aa	5.98 ± 0.01Bb	4.78 ± 0.15Bc	3.16 ± 0.03Bd	<2.0[f]
	2.5	6.45 ± 0.06Aa	4.43 ± 0.09Cb	ND	ND	ND
	3.0	6.45 ± 0.06A	ND	ND	ND	ND
Salmonella	0	6.76 ± 0.07Aa	6.66 ± 0.02Ab	6.25 ± 0.13Ac	5.78 ± 0.11Ad	5.23 ± 0.05Ae
	2.0	6.76 ± 0.07Aa	4.57 ± 0.09Bb	3.74 ± 0.01Bc	3.67 ± 0.05Bd	2.22 ± 0.03Be
	2.5	6.76 ± 0.07Aa	3.75 ± 0.16Cb	2.87 ± 0.07Cc	ND	ND
	3.0	6.76 ± 0.07Aa	<2.0	ND	ND	ND
E. faecalis	0	5.69 ± 0.02Ab	5.95 ± 0.15Aa	5.46 ± 0.02Ad	4.58 ± 0.14Ac	3.66 ± 0.06Ae
	2.0	5.69 ± 0.02Aa	5.17 ± 0.08Bb	4.81 ± 0.12Bc	3.96 ± 0.08Bd	3.29 ± 0.08Be
	2.5	5.69 ± 0.02Aa	4.48 ± 0.04Cb	<2.0	ND	ND
	3.0	5.69 ± 0.02Aa	3.55 ± 0.03Db	ND	ND	ND
S. aureus	0	4.93 ± 0.08Aa	4.36 ± 0.01Ac	4.44 ± 0.00Ab	4.11 ± 0.20Ad	3.98 ± 0.05Ae
	2.0	4.93 ± 0.08Aa	4.02 ± 0.15Bb	3.89 ± 0.02Bc	3.39 ± 0.01Bd	2.10 ± 0.14Be
	2.5	4.93 ± 0.08Aa	3.51 ± 0.03Cb	<2.0	ND	ND
	3.0	4.93 ± 0.08Aa	3.06 ± 0.11Db	ND	ND	ND

ND, not detected after enrichment.
[a]Additive contents of $CaCN_2$ in composting materials in weight percentage.
[b]The mean is the average of six replicates.
[c]The control group that with no $CaCN_2$ mixed.
[d]For each pathogen, means with different upper case letters in a column are significantly different ($P < 0.05$).
[e]Means with different lower case letters in a row are significantly different ($P < 0.05$).
[f]<2.0 log CFU/g, positive after enrichment.

partly related to the unsupportive tendency of high temperature for the growth of microorganisms (Lung *et al.* 2001; Gong 2007). In terms of temperature influence, the inhibitory efficacies of $CaCN_2$ against four pathogens at 20 °C, 30 °C, and 37 °C were more obvious than at 50 °C. The result indicates that it is

Table 4. Log removal for *E. coli*, *Salmonella*, *E. faecalis*, and *S. aureus* during cattle manure composting with addition of different contents of $CaCN_2$ at 30 °C

Microorganism	Additive content[a] (%)	Log removal at sampling time (d)				Average log removal (Mean ± SD)
		1	2	3	5	
E. coli	0[b]	−0.45	−0.31	−0.12	0.41	−0.12 ± 0.38
	2.0	0.47	1.67	3.29	6.45	2.97 ± 2.59
	2.5	2.02	6.45	6.45	6.45	5.34 ± 2.22
	3.0	6.45	6.45	6.45	6.45	6.45 ± 0.00
Salmonella	0	0.1	0.51	0.98	1.53	0.78 ± 0.62
	2.0	2.19	3.02	3.09	4.54	3.21 ± 0.98
	2.5	3.01	3.89	6.76	6.76	5.11 ± 1.94
	3.0	6.76	6.76	6.76	6.76	6.76 ± 0.00
E. faecalis	0	−0.26	0.23	1.11	2.03	0.78 ± 1.01
	2.0	0.52	0.88	1.73	2.4	1.38 ± 0.85
	2.5	1.21	5.69	5.69	5.69	4.57 ± 2.24
	3.0	2.14	5.69	5.69	5.69	4.80 ± 1.78
S. aureus	0	0.57	0.49	0.82	0.95	0.71 ± 0.21
	2.0	0.91	1.04	1.54	2.83	1.58 ± 0.88
	2.5	1.42	4.93	4.93	4.93	4.05 ± 1.76
	3.0	1.87	4.93	4.93	4.93	4.17 ± 1.53

Note: Log removal = $\log N_0 - \log N_x$, $\log N_0$ and $\log N_x$: 0 d and sampling day concentration (log CFU/g). $\log N_x$ in the sampling days in which pathogen was not detected or under the detection limit was taken a value of zero.
ND, pathogen was not detected after enrichment or below the detection limit.
[a]Additive contents of $CaCN_2$ in composting materials in weight percentage.
[b]The control group that with no $CaCN_2$ mixed.

necessary to take full advantage of fungicidal function of $CaCN_2$ during mesophilic composting or at the beginning of composting process, especially in the composting that temperatures could not be uniform throughout the entire mass of compost which is a possibility naturally occurring in all kinds of composting. The temperatures under such conditions are suitable for the growth of microorganisms or at least do not hinder it, so it becomes one of the major difficulties to inactivate pathogens during composting. Several studies have tried to overcome this difficulty (Plachá *et al.* 2008; Avery *et al.* 2009; Wong, Selvam 2009). In the present study, the complete inactivation of four foodborne pathogens during mesophilic composting in the short term was accomplished by addition of $CaCN_2$ into composting materials. However, there was a close relationship between the efficacy of pathogen inactivation and the additive content of $CaCN_2$. In 5 d composting, four tested pathogens all survived at the end of experiment when 2.0% $CaCN_2$ was added into the manure, while they were entirely undetectable from 3 d and 2 d with addition of 2.5% and 3.0% $CaCN_2$, respectively.

Conclusions

1. Survival of *E. coli*, *Salmonella*, *E. faecalis*, and *S. aureus* in the compost piles of fresh cow manure depended on composting temperatures: at thermophilic conditions, their death occurred soon; while at mesophilic conditions, they survived in large populations.

2. With addition of $CaCN_2$, a significant reduction of foodbone pathogens during composting was observed, and the pathogens were completely undetectable in the short term, even under mesophilic conditions.

3. The result of the test indicates that the addition of $CaCN_2$ into manure should be at least 2.5% for complete inactivation of foodborne pathogens during composting.

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