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Effect of Heating Patterns on Inactivation and Regrowth Potential of Bacterial Indicator Organisms in Simulation of Composting

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Abstract

Treatment of sewage sludge by composting for the production of materials useful as a soil amendment is a feasible method of achieving both waste disposal and agricultural enhancement simultaneously. However, the hygienic safety of the final products is an issue which requires resolution. This study was an investigation of the effects of heating patterns on the inactivation and regrowth potential of *Salmonella, Escherichia coli*, and Faecal streptococcus. A laboratory thermal controller was used to apply three patterns of heating; namely, single-impact, constant, and intermittent. Single-impact heating showed the greatest effect on inactivation and intermittent heating had the least effect. Also, the organisms subjected to single-impact heating had the least regrowth potential while those subjected to intermittent heating had the greatest potential. The addition of inorganic suspended solids (kaolin) and dissolved organic matter severely inhibited the inactivation of all the organisms.

Key words: inactivation, regrowth, composting, *E. coli, Salmonella*, Faecal streptococcus, heating pattern

INTRODUCTION

As a method of waste management, composting is preferred to combustion and landfilling and only to source reduction second as a desired practice[1]. Composting is an important mehtod of recycling because it reclaims organic matter[1]. Organic matter is lost from soils by intensive cultivation[2] and composting has economic potential to produce a soil amendment to restore some of the lost organic materials. The government of Japan has announced that utilization of stabilized organic material produced by composting would be one of the top strategies for supporting sustainable agriculture[3].

Although composting is a well developed technology, the potential risk for disease transmission by bacterial pathogens from raw waste must be further considered as a primary concern for public health[2, 4]. The bacteriological safety of compost is usually assessed by testing for indicator bacteria rather than for the pathogens themselves. Heating, which occurs naturally during the composting process, can inactivate both pathogens and indicator bacteria. However, measurement of the bacterial safety of the finished product is complicated by the possibility of bacterial regrowth that can occur as the compost cools[5].

It is known that microbial inactivation during composting is influenced by many factors. Various investigators have studied the effects of temperature, pH, suspended solids, and heating patterns[4, 6-8]. However, no studies addressing the effects of heating patterns on both inactivation and regrowth

have been reported. Peleg *et.al* (2000) reported on the effects of heating patterns on the inactivation of bacterial indicators but did not discuss regrowth potential[8]. Hay (1996) reported regrowth of *Salmonella* sp., in a properly operated composting process[5].

The purpose of this research was to study bacterial inactivation during different patterns of heating that might occur in composting operations followed by evaluation of regrowth rates. The effects adding inorganic suspended solids and dissolved organic matter on inactivation were also investigated.

MATERIALS AND METHODS

Bacterial strains *Salmonella typhimurium* TA 1535[9], *E. coli* K-12, and Faecal streptococcus FS-IFO 33826 were used for the inactivation and regrowth studies. This selection follows previous studies[2, 4, 7, 10-12]. Cultures of the bacteria were grown in sterile LB broth for one day using an incubator shaker (RKc, REX-C900, Japan) at 120 rpm and 28℃. Following the incubation, the cells were harvested by centrifugation at 10,000 × g at 4℃ for 10 minutes and re-suspended with 5 mg/*l* sterile sodium tripolyphosphate buffer (pH 7.0). The centrifugation procedure was repeated once for further washing. Finally, the cells were re-suspended in sterile basal salt medium (BSM)[13] and stored on ice until use.

Sample heating A laboratory thermal controller (GTU-1615, TAITEC) was used for cultivating test samples under various thermal conditions. Composting operations were simulated by subjecting test samples to selected temperature patterns. The controller could either increase or decrease the temperature at a maximum rate of temperature change of 0.7℃/sec. Sixteen samples could be incubated in the controller simultaneously. Preliminary testing confirmed that the heat in the thermal controller was distributed evenly and that the samples were exposed to uniform conditions.

Determination of critical lowest temperature The critical survival temperatures of the test strains were determined in order to find the lowest temperature to be used in the inactivation experiments. Approximately 10^8 cells/m*l* of each test strain were introduced into six sterile 1.5 m*l* tubes and the tubes were incubated at temperatures of 45, 50, 55, 60, 65, and 75℃. During each run, test tubes were removed from the controller at selective time intervals and immediately cooled in iced water. After cooling, the samples were diluted 10-fold with 5-mg/*l* sterile sodium tripolyphosphate (pH 7.0) and 0.1 m*l* of the diluted sample was placed onto the selected medium: Brilliant Green (BG) medium, Desoxycholate medium, and KF medium were used for the strains of *Salmonella typhimurium*, *E. coli*, and Faecal streptococcus, respectively, for detecting survival. Each test was conducted in triplicate.

Evaluation of heating patterns Three model patterns of heating that were considered to simulate composting are shown in Fig. 1. The first pattern consisted of constant heating at a temperature at 65℃ for 20 minutes. The second pattern was single-impact heating, which allowed the temperature of the samples to increase to a maximum of 75℃ for 4 minutes. The third pattern of intermittent heating consisted of an oscillating pattern of temperature changes designed to imitate composting conditions. In this case, the temperature was decreased from 65℃ rapidly to 45℃ to imitate the turning of a pile and then increased back to 65℃. The three heating patterns were designed so that the areas of time-temperature (S_1, S_2, and S_3 in Fig. 1) were the same. In all experimental runs, the initial concentrations of bacteria were approximately 10^8cfu/m*l*. Five tubes, four tubes, and six tubes were prepared for test bacterium under constant heating, single-impact heating, and intermittent heating, respectively. The test organisms were sampled for assessing the rate of inactivation at 0, 5, 10, 15, and 20 minutes for the constant heating pattern; at 0, 4, 8, and 12 minutes for the single impact heating pattern; at 0, 6.6, 11.6, 18.2, 23.2, and 30.0 minutes for the intermittent heating pattern.

During each run, a test tube was removed from the thermal controller at the times selected and immediately cooled in iced water. After the cooling, the sample was

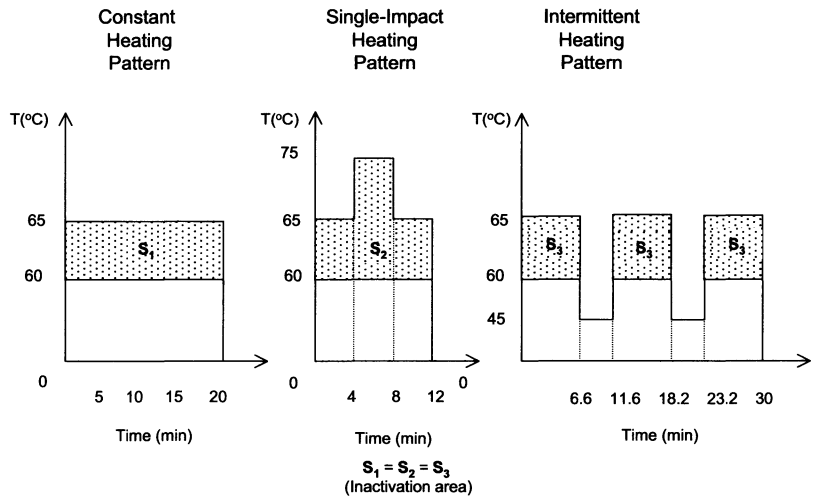

Fig. 1 Heating patterns used in this research.

diluted 10-fold with 5-mg/*l* sterile sodium tripolyphosphate (pH 7.0), and then 0.1 m*l* of the diluted sample was placed onto the selected media: Brilliant Green (BG) medium, Desoxycholate medium, and KF medium for the strains of *Salmonella typhimurium* TA 1535, *E. coli* K-12, and Faecal streptococcus FS-IFO 33826, respectively. All plates were incubated at 37 °C for 24 hrs prior counting the survival population (cfu/m*l*). Each test run was repeated three times.

Evaluation of Regrowth Regrowth is the recovery of indicator bacteria densities to previous relatively high levels after inactivation by heat or chemical disinfectants. Following regrowth indicator bacteria levels may be misleading. Regrowth was assessed by measuring changes in the optical density (OD660 nm) of the samples. Three samples for each indicator organism had set in the previous inactivation experiments were used for evaluation of regrowth. After removal from the thermal controller, a 1-m*l* aliquot of one of the three samples was immediately transferred into a sterile flask containing 200 m*l* of LB broth. The ODs of this flask at time intervals was measured at 37℃. The other two samples were incubated at 37℃. The incubation of one of the two samples was stopped after one hour, a 1 m*l* aliquot was transferred into a sterile flask containing 200 m*l* of LB broth, and the OD of the sample was measured. After two hours, the OD of the last sample was measured following the same manner as above.

Effects of solids and dissolved organic carbon (DOC) The effect by inert suspended solids and DOC on the inactivation of the indicators was investigated. Synthetic sewage sludge was produced by mixing kaolin (Sigma-Aldrich., Japan) into synthetic sewage composed of 8g of meat extract, 12g of peptone, 2g of urea, 5.04g of $Na_2HPO_4 \cdot 2H_2O$, 6g of NaCl, 2.8g of KCl, 3.708g of $CaCl_2 \cdot 2H_2O$, and 4.1g $MgSO_4 \cdot 2H_2O$ in 1 *l* of tap water. The synthetic sewage sludge was autoclaved in flasks, the indicator organisms were added, and the population density was adjusted to approximately 10^8 cfu per gram kaolin. For testing the effects of suspended solids, 0, 10, and 20 % of kaolin solutions were prepared. For testing the effects of DOC, 20, 40, 60, and 80 mg/g of DOC dry weight were prepared.

RESULTS

Lowest inactivation temperatures The inactivation of the bacterial indicators at different temperatures are shown in Fig. 2. The surviving fractions of each of the three indicators showed very similar trends at each temperature and the die-away of all three was more rapid at higher temperatures. At 45 ℃, the decrease in the surviving fraction was

not significant over 60 minutes. At 75℃, the surviving fraction dropped to approximately 0.000,001 in 10 minutes. Although there were significant decreases in the surviving fraction at temperatures of 50℃, 55℃, and 60℃, the rate of decrease was much greater at 65℃ than at 60℃. Therefore, 60℃ was selected as the critical lowest temperature for use in all the following experiments.

Effects of different heating patterns

Survival curves for the bacterial indicators with the three different heating patterns are depicted in Fig. 3. Information on the fraction surviving after a certain heating time is given in Table 1.

For the pattern of constant heating, the trends of the survival curves of the three indicator organisms were very similar. The Faecal streptococcus strain FS-IFO 33826 showed more heat resistance than the other organisms, and the *Salmonella typhimurium* strain TA 1535 had the greatest inactivation with a fraction surviving of only 0.0012 % after 20 minutes (Table 1).

For the single-impact heating pattern, the survival trends were different for the strain of Faecal streptococcus as compared with the other two indicator organisms. It showed a slightly sigmoid or S-shaped curve[14] and more resistance to heat. For the first four minutes of the experiment the die-away rate was low this was followed by a rapid decrease for the next four minutes and then a subsequent decrease in rate to the end of the experiment. The curves for *Salmonella typhimurium* and *E. coli* were nearly exponential (first order decay patterns) although the survival curve for *E. coli* was

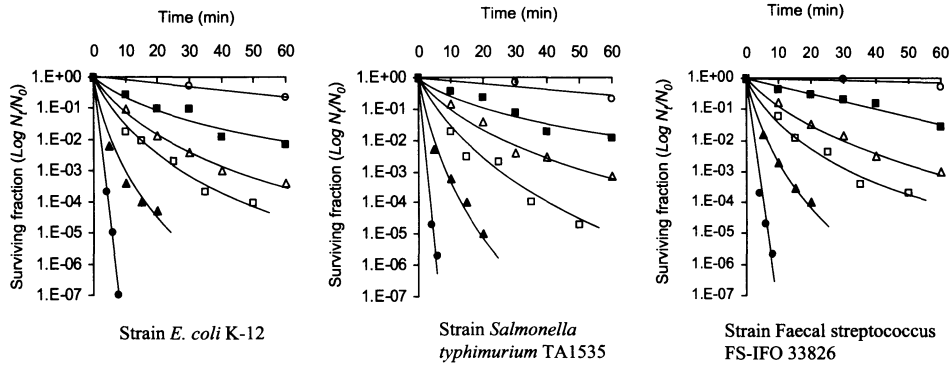

Fig. 2 Survival curves of the bacterial indicator organisms (constant heating).
N_0: initial population; N_t: population at time t
○:45℃, ■:50℃, △: 55℃, □: 60℃, ▲: 65℃, ●: 75℃

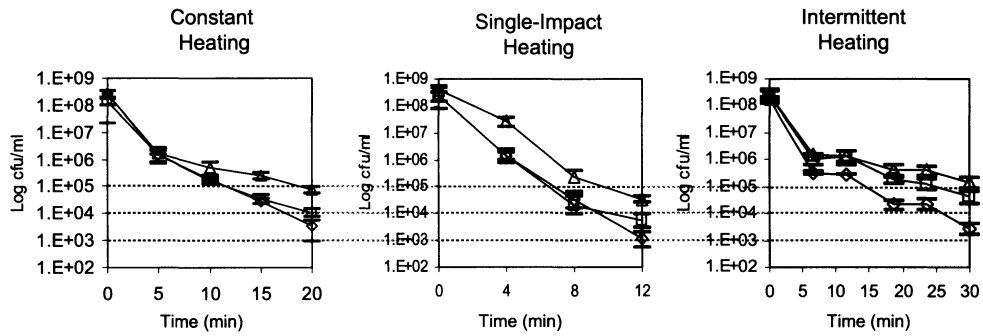

Fig. 3 Effect of the different heating patterns on the inactivation of the bacterial indicator organisms.
□:Strain *E. coli* K-12, ◇:Strain *Salmonella typhimuium* TA1535,
△: Strain Faecal streptococcus FS-IFO 33826.

Table 1 Survival of the bacterial indicator organisms by the different heating patterns

Mode of heat inactivation	Exposure Time, t (min)	Strain *E. coli* K-12		Strain *Salmonella typhimurium* TA1535		Strain Faecal streptococcus FS-IFO 33826	
		N_t*	(N_t/N_0)%	N_t*	(N_t/N_0)%	N_t*	(N_t/N_0)%
Constant Heating	0	1.33×10^8	0.0078 (±0.0024)	2.57×10^8	0.0012 (±0.0009)	2.70×10^8	0.0270 (±0.008)
	20	1.04×10^4		3.25×10^3		7.30×10^4	
Single-Impact Heating	0	2.47×10^8	0.0023 (±0.0011)	2.33×10^8	0.0005 (±0.0002)	4.00×10^8	0.0083(±0.0022)
	12	5.67×10^3		1.20×10^3		3.33×10^4	
Intermittent Heating	0	2.50×10^8	0.0175 (±0.0082)	1.70×10^8	0.0016 (±0.0007)	2.63×10^8	0.0547 (±0.0221)
	30	4.37×10^4		2.73×10^3		1.44×10^5	

* N_t: Number of the indicator density at the time t (N_0: number at time zero). All numbers are mean values.

nearly asymptotic after eighth minutes. The single-impact heating showed the highest effectiveness in terms of inactivation and all indicator organisms reached a population level of less than 10^5cfu/m*l* in twelve minutes.

The trends of the survival curves with intermittent heating which imitates the turning operations of a compost pile followed the typical sigmoid decay pattern[14] (Fig. 3). Two lag phases were seen, from 6.6 minutes to 11.6 minutes and from 10.2 minutes to 23.3 minutes. The temperature of the lag phases was at 45℃ and, according to the results in Fig. 2, the three indicator organisms experienced almost no inactivation effect at 45℃. It seems clear that the pronounced reduction of the rate of inactivation at 45℃ caused the lag phases. This heating pattern showed the lowest inactivation effects on the three indicator organisms. Although more time was provided for the die-away, the survival percentages were the highest of the three heating patterns and the strain Faecal streptococcus FS-IFO 33826 still remained at the population level of 10^5cfu/m*l* after 30 minutes (Table 1).

Regrowth of the bacterial indicator organisms The regrowth curves for strain *E. coli* K-12 are shown in Fig. 4. The curves for the strains *Salmonella typhimurium* TA 1535 and Faecal streptococcus FS-IFO 33826 were also obtained but they are not presented because they were almost identical with that of *E. coli* K-12. The samples which were incubated at 37℃ for two hours grew first and the samples which were transferred from

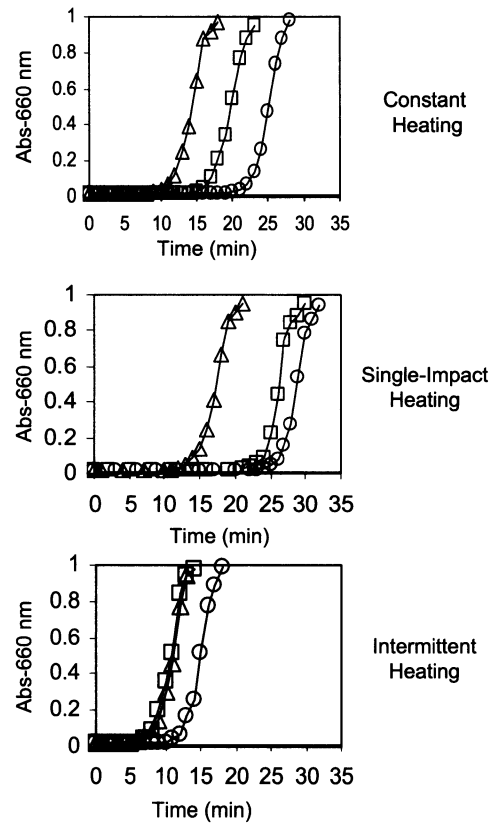

Fig. 4 Regrowth curves of strain *E. coli* K-12 at 37℃ after the three thermal inactivation.
○:right after inactivation,
□:after 1 hour-incubation at 37℃,
△:after 2 hour-incubation at 37℃

the thermal controller without incubation showed the longest lag for prior to regrowth. The regrowth of the samples incubated for one hour and two hours yielded virtually the same curve for the intermittent heating pattern and the recoveries were the most rapid. The regrowth curves for the single impact heating showed the slowest recovery and this corresponds with the efficiency of inactivation of the bacterial indicator organisms. High efficiency of inactivation of the indicator organisms led to slow regrowth of the indicator organisms.

Effects of solids The results of experiments evaluating the effect of solids on thermal inactivation are shown in Fig. 5. The trends of inactivation were similar for all three the heating patterns and bacterial indicators. The highest inactivation was found for 0 % kaolin and the lowest inactivation was shown for both 10 and 20 % kaolin.

Effect of DOC Experiments to evaluate the effect of DOC concentration were carried out only with a constant heating pattern with 20% kaolin concentration used for all experiments. According to the results shown in Fig. 6, two trends were found. One trend

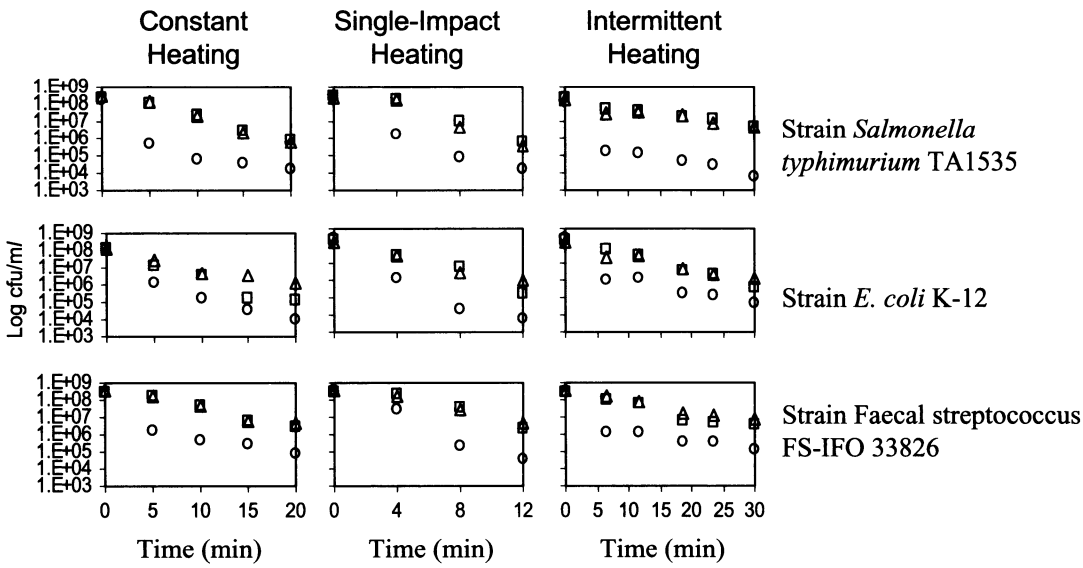

Fig. 5 Effect of solids on thermal inactivation.
Kaolin contents: ○:0 %, □:10%, △:20%

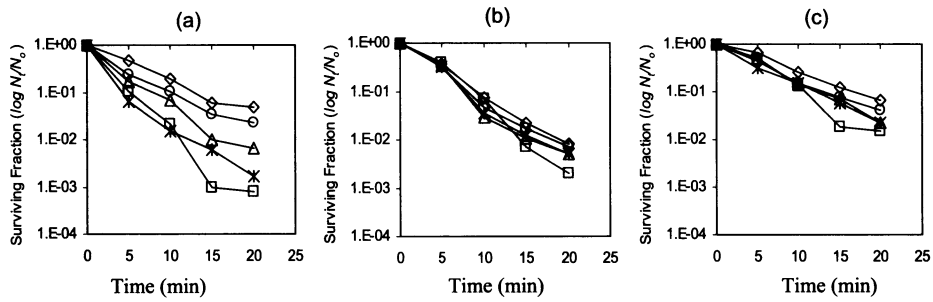

Fig. 6 Effect of different concentration of D℃ (@ 20% Kaolin).
□:0 mg/g, ＊:20 mg/g, △:40 mg/g, ○:60 mg/g, ◇:80 mg/g (dry base)

was for the strain *E. coli* K-12; inactivation strongly depended on the concentration of DOC. The inactivation of the strain *E. coli* K-12 was the greatest when the DOC concentration was the least. The other trend was the same for the strains of *Salmonella typhimurium* TA 1535 and Faecal streptococcus FS-IFO 33826; inactivation was not influenced by the concentration of DOC.

DISCUSSION

Composting operations are variable. Some operations require turning of piles but others do not. Some composting operations use temperature control to prevent the temperature from reaching the allowable maximum but others allow the temperature to reach a maximum. The effects of heating patterns on inactivation of pathogens and their regrowth potentials were studied to provide a basis for composting properly in terms for inactivation of bacteria associated with humans. The results showed that single-impact heating was the most effective for inactivation and had the greatest effect on regrowth. It can be said that, from a hygienic point of view, composting should be operated without temperature control to allow the temperature to increase to the maximum. Intermittent heating showed the lowest effect because there were two periods during which the temperature was far below the critical inactivation temperature although the total inactivation exposures were the same for all three heating patterns. As shown in Fig. 4, the indicator organisms treated under the intermittent heating pattern regrew approximately 1.5 to 2.1 and 1.7 to 3.2 times faster than those treated under the constant heating and the single-impact heating patterns. This also indicates that inactivation by intermittent heating was less effective than by the other heating patterns. It can be concluded that turning piles during composting operations is not beneficial for inactivation of microorganisms.

The effect of solids on inactivation of the bacterial indicator organisms was also investigated. In the range of kaolin content from 0 to 20%, the presence of solids inhibited the inactivation and enhanced the survival of the indicator organisms and the rate was almost the same when the solids content was 10 and 20% regardless of the indicator organisms and the heating patterns (Fig. 5). Ugwuanyi *et.al.* (1999)[5] also reported the effect of suspended solids on the inactivation of *E. coli*. Their results showed that a decrease in the survival rates occurred with an increase in suspended solids content from 2 to 8%. However, the results presented in this paper show an decrease in the survival rate at suspended solids concentrations of 10 % and 20%.

Adding DOC to 20% kaolin solution did not inhibit inactivation of *Salmonella typhimurium* TA 1535 and Faecal streptococcus FS-IFO 33826. However, the inactivation of *E. coli* K-12 was clearly influenced the DOC and the surviving fraction decreased with an increase of the DOC concentration. Consequently, sewage sludge which contains suspended solids and DOC can influence the inactivation of the bacterial indicator organisms in composting operations. The inactivation and regrowth of the indicator organisms in the solid phase under different heating patterns should be investigated in future studies.

CONCLUSIONS

It was found that turning of compost piles during composting should be minimized, from the hygienic point of view, and that achieving higher temperatures in the piles results in more effective inactivation of the bacterial indicator organisms. As Tateda *et.al.* (2002) reported, very uneven temperature distributions would be expected if turning is not conducted during composting[15]. Therefore, effective and optimal turning schemes should be investigated by minimizing the number of turnings during operation.

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