Original Article

Inactivation Kinetics of Indicator Microorganisms during Solar Heat Treatment for Sanitizing Compost from Composting Toilet

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Abstract

This study aimed to estimate the sanitization effectiveness of compost by solar heating. Compost produced from composting toilet was inoculated with *Escherichia coli*, *Enterococcus faecalis* and *Ascaris* eggs and subjected to solar heating. The heating was performed by direct exposure of compost to sun and in a solar box. From treated compost, the number of isolated bacteria was determined by plating method and the number of *Ascaris* eggs was determined by microscopy counting. The inactivation kinetics of microorganisms were modeled using nonlinear regression software tool. The result showed that the temperature regime produced by direct sunlight and solar box during heating were categorized as mesophilic ($> 30^{\circ}\text{C}$) and pasteurization ($> 70^{\circ}\text{C}$), respectively. The log reduction of microorganisms in heated compost by solar box was significantly higher than that of direct sunlight. The inactivation rate was slow in compost heated by the sun but fast in compost heated by solar box. *Escherichia coli* appears to be the most sensitive to destruction temperatures achieved by solar heating. The thermal decimal decay occurred rapidly in solar box while it was prolonged with the direct sunlight. The high and uniform temperature distribution obtained with solar box during heating proved to be an efficient option for safe use of compost.

Keywords: sanitizing compost, composting toilet, solar heating

INTRODUCTION

Serving over 3 billion people, mainly in low- and middle-income countries, on-site sanitation is still the main solution for the majority of the world's population for sanitizing human excreta [1]. However, excreta management remains a big issue in these countries where some part of the fecal matter is used without treatment as fertilizer [2]. Indeed, toilet waste fractions contain a major proportion of nutrients from households [3] which are associated with a high risk of pathogen content and have to be disinfected before use as fertilizer or soil conditioner. Producing a safe fertilizer from human feces enables to break the fecal-oral transmission route via emission to the recipient water and contaminated food. For avoiding this transmission along the food chain, contamination has to be broken as early as possible.

Common technologies applied in sanitizing for treatment

of feces include the use of different types of latrines, septic tanks, and dry or composting toilets [4]. The most common treatment is composting, where the main inactivation of pathogens is due to increased temperature [5]. However, in most cases, the temperature of composting toilet in developing countries, do not greatly increase to destroy all pathogens [6]. Accordingly, the practice of adding inert materials like ash and soil in dry toilets may hamper heat generation in the compost [7]. Even with heat generation, maintenance strategies, such as turning the compost during high temperature periods, are necessary to ensure inactivation of the entire volume of the matrix [8]. Low-temperature degradation of fecal matter, i.e. storage, requires a treatment time exceeding a year [5] and even with that the product cannot be considered as pathogen-free.

One of the most reliable methods for sanitizing compost is heating. The unconventional, low-temperature technology

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of solar heating of fecal material is a prospect from the economic and ecological point of view [9]. Due to sanitary and epidemiological reasons, it is necessary to establish microbiological methods which will allow constant monitoring of compost sanitization effectiveness in solar heating process. To reach high and efficient temperatures for the inactivation of pathogens in full compost, heating has to be accumulated and preserved for a long time to achieve appropriate disinfection [10]. Considering the idea of using the cheapest and available energy for fast thermal sanitizing, solar energy is an alternative to increase heating efficiency [11]. Using a solar box, a simple and low-cost heating system available locally in Burkina Faso, compost can be heated at a sufficient temperature to destroy most of the pathogens in a few hours without burning the organic matter. Solar box can concentrate sunlight at high intensity and then be used for inactivating pathogens in compost.

Several studies report the sanitizing of human feces [3], manure [12] and sludge [13] by thermal treatment, but the effectiveness of these systems on compost has not been studied yet. The lack of information about solar heating in tropical conditions indicates the necessity for further studies on the processes and questions the promotion of sanitation in a safe way. Investigation on the possibility of applying a new approach in sanitizing fecal material could shorten the storage time and minimize handling of compost. Inactivation rate or the degree of decay can be used to assess and compare the quantitative effectiveness of a treatment process on pathogen destruction [14]. This study aimed to estimate the sanitization effectiveness of solar-heated compost by comparing the sun and solar box.

MATERIALS AND METHODS

Compost collection

Compost used for the experiments was collected from a urine-diverting composting toilet prototype installed in a pilot household located in Ouagadougou, the capital city of Burkina Faso. This toilet coupled with an aerobic composting reactor was used by 8 – 10 persons with a total of 6 to 8 kg of compost produced for approximately 2 months. The matrix used as bulking agent was sawdust and there was no chemical source addition (ash or lime) as alkaline treatment. Compost samples were collected and stored at 4°C.

Collection of microorganisms and suspension preparation

Compost samples were inoculated by selected indicator

bacteria and parasite eggs in order to assess the entire inactivation process. The bacterial strains, *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212, used in this study were purchased from the American Type Culture Collection (ATCC, Manassas, USA). For suspension preparation, each bacteria, *E. coli* and *E. faecalis*, were cultured in a growth medium Nutrient Broth (BD, Franklin Lakes, USA) and incubated in a shaking water bath at 37°C overnight. The bacterial suspensions were used as a source of inoculums. The parasites used were *Ascaris lumbricoides* eggs which were extracted from human feces collected from patients at a hospital in Ouagadougou, Burkina Faso. According to the method of Collick *et al.* [15], the feces collected were washed through a series of sieves ranging from 425 to 38 µm. Eggs were finally collected on the 38-µm sieve. The eggs were stored in a 4°C refrigerator in reverse osmosis water and 10% formaldehyde to prevent mold growth. Upon use, a portion of the egg solution was collected as a source of inoculums.

For compost sample inoculation, an adequate aliquot of *E. coli* (0.3 mL about 10^7 CFU/mL), *E. faecalis* (0.3 mL about 10^7 CFU/mL) and *Ascaris* eggs (1 mL about 10^3 eggs) was added to 1,000 g of autoclaved (121°C) compost. The concentrations of microorganisms added were normal concentrations found generally in feces in the area of this study. Before subjecting to solar heating, the moisture content of compost sample was adjusted to 20% (w/w) with sterilized deionized water.

Solar heating treatment set up

The solar heating experiment was carried out at 2iE University campus located in Ouagadougou City, latitude 12°17.5' and longitude 1°7'. Compost samples were subjected to heating by exposing them directly to the sun and placing in a solar box, resulting in two platforms of treatment. As control, the same amount of compost was kept at 4°C in laboratory throughout the experimental period. In each treatment platform, the compost was spread out in a conical shape of 40 cm diameter and 30 cm height.

For direct sun exposure, the platform was made up of wood with a size of 85 cm length × 40 cm width. The solar box used (**Fig. 1**) had a size of 85 cm length × 40 cm width × 30 cm height at the front cover and × 40 cm height at the back cover. It can provide temperatures for heating by combining the technique of solar concentration rays and greenhouse effect. The box had an absorbing plate surface of 0.54 m² and a thermal conductivity of 80 W/(m·K). The insulating material was made with wood, plywood, kapok wool, cotton wool and thick reflective paper. The solar box

Fig. 1 Solar box used.

was provided by the Laboratory of Solar Energy and Energy Savings of the same university.

Sampling

The compost samples subject to heating and the control compost were sampled at every hour interval during 5 hours. The top layer of compost was removed with a hand trowel; one scoop of compost was collected and placed in a sterilized bucket. The procedure was repeated for a total of three grab samples for each sampling depth: top, middle and bottom. The three grab samples were mixed thoroughly in the bucket with the hand trowel and a subset of the mixed sample was transferred to a sterilized bottle. The bottle was sealed and placed in an insulated cooler with ice. The composite sample (30 g) was assayed for microbiological and physicochemical analyses.

Microbiological analysis

For bacterial extraction, a 3% (w/v) beef extraction solution was used. Ten grams of compost sample was added to a 90-ml volume of extracted solution and agitated for 3 minutes to extract bacteria. After adequate dilution (7 fold) with phosphate buffer, each extract was inoculated by double-layer method in Chromocult coliform agar ES (BD, Franklin Lakes, USA) for *E. coli* and Slanetz-Bartley agar (BD, Franklin Lakes, USA) for *E. faecalis*. These inoculated media were incubated at 37°C for 24 h for *E. coli* colony count and at 37°C for 48 h for *E. faecalis* colony count. The detection limits of bacteria were 10 CFU/g and the concentration

of bacterial count was expressed in CFU/g of dry compost.

For parasite analysis, 10 g of compost sample was homogenized with 90 mL of 0.1% Tween 80 for 1 min using a blender and screened through 4 layers of folded wet gauze. The filtrate was collected in round-bottom flasks and allowed to settle for 3 hours. The number of *Ascaris* eggs was determined by the concentration method followed by a direct count under optical microscope B-350 (OPTIKA, Ponteranica, Italy), described by Sossou *et al.* [6]. The viability of *Ascaris* eggs was assessed both by morphological analysis for integrity (normal cellular structure) and staining through dye exclusion method using Safranin O. If the dye had penetrated into the eggs, they were counted as nonviable. The detection limits of *Ascaris* eggs were one egg/g and the number of *Ascaris* egg count was expressed as number of eggs/1,000 g of dry compost.

Physicochemical analysis

Compost temperatures at different depths: top, middle and bottom, were recorded using a portable multi-logger ZR-RX45 (OMRON, Tokyo, Japan) at every hour interval from 11:00 a.m. to 4:00 p.m. local time. The pH was measured in 1:5 water extract with a Hanna Digital Compo Meter HI991405 (Hanna Instruments Ltd, Leighton Buzzard, UK). Free ammonium concentration was determined using micro-Kjeldahl distillation method. Moisture and ash content were determined through the weight loss at 105 and 550°C, respectively, by the procedures established in the standard methods [16].

Climatic condition monitoring

The solar heating experiment was carried out in September during the summer period and there was no rainfall throughout the experimental day. Meteorological data of the experimental day, such as air temperature, insolation and humidity, were taken from Burkina Faso Meteorological Station.

Data analysis

During the solar heating experiment, temperature and other environmental factors affecting microbial inactivation were not constant. Therefore, the Geeraerd and Van Impe Inactivation Model Fitting Tool (GInaFiT) version 1.6.1 was used for testing microbial inactivation models best fitting our data [17]. The best-fitting models were the log-linear regression for *E. coli* and *E. faecalis*, and the log-linear regression plus shoulder for *Ascaris* eggs. All models were run for each inactivation curve and the values of the root-mean-square error (RMSE) were compared. The RMSE is considered to be the simplest and most informative measure of goodness-of-fit for linear and nonlinear models [17]. The model with the smallest RSME was considered the best fit for the respective inactivation curve. When the magnitude of RMSE was much smaller than the experimental precision, the model was considered to over-fit the data and a simpler model was chosen. If two models had the same or very similar RMSE values, the simpler model was considered to fit best. The maximum inactivation rate constant k_{max} (1/h) and the decimal decay T90 values (time to inactivate 90% of the population) were calculated using the best fitting model in GInaFiT. The kinetics destruction by log-linear regression and log-linear regression plus shoulder were expressed respectively as follows:

$$N(t) = N(0) \cdot e^{-k_{max} \cdot t} \quad (1)$$

$$N(t) = N(0) \cdot e^{-k_{max} \cdot t} \cdot \frac{e^{k_{max} \cdot SI}}{1 + (e^{k_{max} \cdot SI} - 1) \cdot e^{-k_{max} \cdot t}} \quad (2)$$

where $N(t)$ and $N(0)$ expressed in CFU or number of eggs/g of dry compost are the concentrations of microorganism in compost at time t and 0, respectively; and t is the reaction time (h). The parameter used for the estimation by GInaFiT was SI (shoulder length i.e. the length of the lag phase) (h), which represents the degrees of freedom. The k_{max} values and standard deviation were calculated directly by GInaFiT program.

All results were expressed as the mean values of three independent experiments. Log_{10} transformed microorganisms'

counts were subjected to one-way and two-way analysis of variance (ANOVA) to compare the effects of the type of treatments (control, sun and solar box) and the duration time of treatment. The one-way ANOVA was also applied on physicochemical parameters of compost before and after treatment. Treatment means were compared with the least significant differences test at $\alpha = 0.05$. These statistical analyses were performed using StatView Software version 5.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Parameter monitoring during solar heating

The sanitization effectiveness of compost by solar heating was closely associated with weather conditions. During the day of the experiment, from 11:00 a.m. to 4:00 p.m., the average daily level of solar radiation was 598.4 W/m^2 , and the maximum radiation recorded reached the value of 610 W/m^2 . The relative humidity was dependent on the air humidity and showed considerable fluctuations; the lowest value was 32%, the highest was 64%, and the average was 48%.

Figures 2 and 3 showed the change of ambient temperature and temperature at different depths of compost pile during direct sunlight and solar box heating. The results showed that, the ambient temperature varied from 35.5°C to 44.5°C , with the average value of 38.4°C . During direct sunlight heating, the recorded temperatures at each depth of compost pile reached their maximum after 5 hours and were 47, 44, and 41°C at the top, middle, and bottom, respectively. On the other hand, during solar box heating, the recorded temperatures at each depth reached their maximum after 3 hours and were 76, 72, and 70°C at the top, middle, and bottom, respectively. As expected, the increase of compost temperatures during solar heating was significantly correlated with the duration of heating process. The solar box heating significantly increased the temperatures at each depth of the compost pile than direct sunlight heating. Such results highlighted that rapid inactivation temperatures above 55°C could be reached only with the solar box since direct sunlight heating never reach this minimum temperature. Furthermore, in the solar box, the temperature profiles at different depths showed similar values indicating a uniform temperature distribution in the compost pile during heating. The recorded temperatures in the compost control were slightly below those of ambient temperature.

Fig. 2 Ambient temperature and temperature in compost during direct sunlight heating.

Fig. 3 Ambient temperature and temperature in compost during solar box heating.

Table 1 Physicochemical property change during compost heating.

Parameters	Before treatment	After treatment		
		Control	Direct sunlight	Solar box
pH	7.64 (0.05)*	7.61 (0.23)	7.49 (0.02)	7.43 (0.72)
Free ammonium (mg/L)	190 (7.01)	189 (6.3)	173 (5.9)	161 (5.4)
Moisture content (%)	20 (0.91)	19.59 (0.84)	12.97 (0.69)	10.02 (0.63)
Ash content (%)	12 (0.08)	11.83 (0.07)	10.14 (0.06)	9.81 (0.05)

*Data in parentheses are standard deviations

Physicochemical property change of compost during heating

The physicochemical property change of compost during heating is presented in **Table 1**. The pH in compost decrease from 7.64 to 7.49 for direct sunlight heating and to 7.43 for solar box heating. The concentration of free ammonium in compost decreased slightly from 190 to 173 mg/kg for sun heating and to 161 for solar box heating. The reduction of free ammonium concentration is probably due to its conversion and its volatilization as ammonia. On the other hand, the loss of this ammoniacal nitrogen by heating temperature is responsible for the pH reduction. The moisture content in the compost was 20% before heating but it decreased to 12.97% after direct sunlight heating and to 10.02% after solar box heating. This reduction corresponding to a loss of water in compost during heating would indicate a drying effect during solar heating. The ash content showed minimal losses of 1.86% and 2.19% during direct sunlight heating and solar box, respectively. For all physicochemical parameters analyzed, there was no significant change in values during heating indicating that these parameters did not have enough effects on microorganisms' inactivation.

Change of indicator microorganisms during solar heating

The change of the number of *E. coli*, *E. faecalis* and *Ascaris* eggs during solar heating are presented in **Figs. 4, 5 and 6**, respectively. The results showed that solar heating significantly reduced the number of microorganisms tested.

The change of *E. coli* and *E. faecalis* population in compost showed a linear curve of regression. After 5 hours of heating, the number of *E. coli* decreased to an average of about 1.6 log reduction for direct sunlight heating and about 3.2 log reduction for solar box heating. At the same time, the number of *E. faecalis* isolated from compost decreased to an average of about 0.8 log reduction for direct sunlight heating, and by about 1.6 log reduction for solar box heating. A linear regression analysis indicated that there was a significant decay in the log numbers of *E. coli* and *E. faecalis* during solar heating and in relation with exposure duration. The inactivation curves for *Ascaris* eggs showed two phases: a lag phase as an initial phase followed by a rapid decrease corresponding to shoulder and log-linear decay. The reduction in the *Ascaris* population during solar heating was about 0.2 log reduction with direct sunlight heating and about 0.8 log reduction with solar box heating. The nonlinear regres-

Fig. 4 Change of *E. coli* during solar heating. Each data point represents a mean value $\pm$ standard deviation.

Fig. 6 Change of *Ascaris* eggs during solar heating. Each data point represents a mean value $\pm$ standard deviation.

Fig. 5 Change of *E. faecalis* during solar heating. Each data point represents a mean value $\pm$ standard deviation.

sion of *Ascaris* eggs indicated that there was a significant decay in the log numbers during solar heating and in relation with exposure duration. For all microorganisms tested, the number detected in treated composts declined more rapidly in compost heated by solar box than that of direct sunlight.

Inactivation rate and decay of microorganisms during solar heating

The estimated k_{max} and T90 of microorganisms determined during solar heating are presented in **Table 2**. The k_{max} of *E. coli*, *E. faecalis* and *Ascaris* eggs in compost heated by direct sunlight were 0.82, 0.39 and 0.34 1/h, respectively while in compost heated by solar box were 1.55, 0.84 and 0.73 1/h, respectively. This indicated that the inactivation rate was slower in compost heated by direct sunlight but fast in compost heated by solar box. The T90 values of *E. coli*, *E. faecalis* and *Ascaris* eggs in compost heated by direct sunlight were 1.22, 2.56 and 2.94 hours, respectively while in compost heated by solar box were 0.65, 1.19 and 1.37

hours, respectively. This revealed that the thermal decimal decay occurred rapidly in solar box while it was prolonged with the sun. The effective significant difference of k_{max} and T90 values was observed between compost heated by solar box and that of direct sunlight. For all microorganisms tested, k_{max} was reduced while T90 values were prolonged with temperature decrease. These results demonstrated the positive effects of solar heating on microbial inactivation.

DISCUSSION

The experimental protocol described in this study was designed to measure the instantaneous effects of different time-temperature exposure to solar heating on the survival of microorganisms. The results of temperature increase during the experiment showed that the tested temperature conditions were categorized into mesophilic ($> 30^{\circ}\text{C}$) and pasteurization ($> 70^{\circ}\text{C}$) for direct sunlight heating and solar box heating, respectively [14]. Furthermore, recorded temperatures in the compost placed in the solar box showed similar values at different depths. This indicated a uniform temperature distribution in the compost pile during solar box heating. The elevated temperature during direct sunlight and solar box heating should show an effect on pathogen destruction. Indeed, solar heating can effectively destroy pathogens in fecal material when raising the temperature [18].

During compost heating with direct sunlight at mesophilic conditions for 5 hours, only a slight reduction (1.1 – 2.1 log reduction) of microorganisms was observed, indicating a low sanitizing effect. This low sanitization effectiveness of direct sunlight heating was confirmed by the results obtained by Mathioudakis *et al.* [19]. Similar to these results, Bux *et*

Table 2 Estimated maximum inactivation rate constant (k_{max}), decimal decay rate (T90) and root-mean-square error (RMSE) of regression lines for microorganisms.

Treatment	<i>E. coli</i>			<i>E. faecalis</i>			<i>Ascaris</i> eggs		
	$k_{max}(1/h)$	T90 (h)	RMSE	$k_{max}(1/h)$	T90 (h)	RMSE	$k_{max}(1/h)$	T90 (h)	RMSE
Control	0.47 ^a	2.13 ^a	0.0494	0.38 ^a	2.63 ^a	0.0708	0.26 ^a	3.85 ^a	0.0013
Direct sunlight	0.82 ^b	1.22 ^b	0.1500	0.39 ^a	2.56 ^a	0.0830	0.34 ^a	2.94 ^a	0.0027
Solar box	1.55 ^c	0.65 ^c	0.3318	0.84 ^b	1.19 ^b	0.1252	0.73 ^b	1.37 ^b	0.006

Values followed by different alphabetical letter (“a”, “b” and “c”) are significantly different for $p < 0.05$.

al. [20] and Ögleni and Özdemir [21], have obtained a low degree of elimination of fecal pathogens, not exceeding 1 log reduction with direct sunlight heating. The optimum cardinal temperature for growth and survival of enteric organisms is in the range of 30 – 40°C [14]. Therefore, mesophilic temperatures could not exert a specific thermal stress on the decay of microorganisms. During compost heating with solar box at pasteurization conditions for 5 hours, a significant reduction (2.1 – 3.1 log reduction) of microorganisms was observed. In similar conditions (above 55°C), Aitken *et al.* [22] and Popat *et al.* [23] reported > 3 log reduction of pathogens. Pasteurization temperatures ranging from 65 to 80°C are very effective in pathogen inactivation [24].

As expected for each microorganism tested (*E. coli*, *E. faecalis* and *Ascaris* eggs), the maximum inactivation rate constant was significantly low (0.82; 0.39; 0.34 1/h, respectively) during direct sunlight heating but significantly high (1.55; 0.84; 0.73 1/h, respectively) during solar box heating. So, the pasteurization conditions (> 70°C) by solar box heating showed a rapid inactivation of microorganisms than the mesophilic conditions (> 30°C) by direct sunlight heating. The inactivation of microorganism with increasing temperature and treatment duration are well defined. A thermophilic temperature higher than 55°C is sufficient to destroy all enteric organisms, whereas mesophilic conditions extend treatment duration [14]. For that, 55°C is a rapid and minimum required temperature for pathogen inactivation [25]. Indeed, most of the microorganisms could survive well at low temperatures but rapidly die off at temperatures above 55°C [26]. When temperatures in compost pile reached as high as 55°C or above, this place microbial populations present in great stress which become lethal for them along time [27]. Aitken *et al.* [28] studied the inactivation of *E. coli* in manure and reported a mean inactivation rate constant (k) range in 13.8 – 15 1/h at 55°C. In our study, we reported an inactivation rate constant of 1.55 1/h at temperature > 70°C for *E. coli* strain. In the same way, Aitken *et al.* [22] and Popat *et al.*

[23] found for *Ascaris* eggs in sludge at 55°C, an inactivation rate constant of 13.30 1/h and 3.05 1/h, respectively, while in our study we found 0.73 1/h at temperature > 70°C. The inactivation rate constants of microorganisms in compost observed during solar heating in this study were significantly less than those of previous reports. This is probably due to the difference of the fecal matrix.

The die-off time is usually expressed as a decimal decay (T90) or as the time after which organisms are no longer detectable. The maximum survival time of *E. coli*, *E. faecalis* and *Ascaris* eggs during solar box heating was 0.65, 1.19 and 1.37 h, respectively, and during direct sunlight heating was 1.22, 2.56 and 2.94 h, respectively. Turner [25] conducted a bench-scale study of the inactivation of *E. coli* in sterilized materials and found that at 55°C, *E. coli* was consistently reduced to below detection limits within 2 h. For enterococci, Pike *et al.* [29] reported that the T90 was 31 min in a laboratory-based inactivation experiment with sludge at thermophilic temperatures. At temperatures above 65°C, *Ascaris* egg is destroyed in compost in 1 hour [30]. This indicates that only the solar box provided a decimal decay which approached those found by previous authors. However, the solar box heating was not effective for reducing all the microorganisms' population in the compost. The remainder of *E. coli*, *E. faecalis* and *Ascaris* eggs concentration in the compost after 5 hours solar box heating was 2.47×10^3 CFU/g, 1.14×10^4 CFU/g and 65 number of eggs/g, respectively. WHO reported in the guidelines for verification monitoring in large-scale treatment systems of excreta (compost in our case) that the product is considered safe for use when there is less than 10^3 *E. coli*/g and one helminthes egg/g of dry solid [5]. Thus, the compost heated in the solar box has not yet met the acceptable levels of WHO [5]. Based on the inactivation kinetics of microorganism obtained in this study and considering 5 hours as a daily optimal treatment, total inactivation of *E. coli*, *E. faecalis* and *Ascaris* eggs occurred in the solar box for about 3, 5 and 3 days, respectively. How-

ever, this study has not been done in a large-scale. Therefore, care might be taken when applying solar box heating on a large amount of compost.

Comparing the log reduction, k_{max} and T90 values, *E. coli* was more affected by the solar heating than *E. faecalis* and *Ascaris* eggs. This is consistent with previous inactivation temperature studies where *E. coli* was more sensitive to heat inactivation [31]. Thus, *E. faecalis* and *Ascaris* eggs were resistant. Previous enterococci monitoring studies had also reported the sigmoidal inactivation of enterococci [32], indicating the resistance of this bacteria to adverse conditions [33]. Besides, *Ascaris* eggs were more resistant to thermal inactivation than other pathogens and are considered the most environmental resistant pathogens excreted in human feces [5,34].

Thermal destruction of pathogens may well depend on factors other than temperature, i.e. pH, moisture content, free ammonia concentration and duration of treatment, which may enhance pathogen inactivation [25]. In this study, there was no significant change of physicochemical parameters during heating. Thus, this would indicate that the change of physicochemical parameters did not have effects on microorganisms' inactivation. Therefore time-temperature inactivation was the main process responsible for microorganism decay during solar heating. Exposure temperatures are identified as the principal factor influencing pathogen inactivation in a fecal material [35]. However, Aitken *et al.* [28] and Popat *et al.* [23] reported that the impacts of physicochemical parameters on the inactivation kinetics during heating should exist, but undiscovered.

The decay mechanism of enteric organisms is well defined by time-temperature inactivation. It is generally demonstrated that the increase of temperature has a direct impact on bacterial decay due to the autolysis of bacterial cells [14,36]. Although the primary inactivation mechanism of *Ascaris* eggs at high temperatures is not well known, Aitken *et al.* [22] and Popat *et al.* [23] speculated that the primary inactivation mechanism of *Ascaris* eggs could be protein capsid denaturation.

Finally, quantification of bacteria in this study was enumerated using a conventional culture method. Although some reports indicated the correlation between culture and direct count method [32], there is still a need to validate the culture method to the viable cells and not cultivable cells. In the same way, Collick *et al.* [15] reported about parasite eggs that to ascertain their viability in stress conditions, count by larvae development is needed compared to direct microscopy count which was used in this study.

CONCLUSIONS

This study investigated the sanitization effectiveness of compost from composting toilet by comparing direct sunlight and solar box heating. The results showed that the temperature regime produced by direct sunlight and solar box during heating were categorized in mesophilic ($> 30^{\circ}\text{C}$) and pasteurization ($> 70^{\circ}\text{C}$), respectively. The log reduction of microorganisms in heated compost by solar box was significantly higher than that of the sun. The inactivation rate was slower in compost heated by direct sunlight but fast in compost heated by solar box. *E. coli* appears to be the most sensitive to destruction temperatures achieved by solar heating. The thermal decimal decay occurred rapidly in solar box while it was prolonged with direct sunlight. The high and uniform temperature distribution obtained with solar box during heating proved to be an efficient option for safe use of compost.

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