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Effect of Thermal Treatment on the Disinfection of Infectious Waste by Fry-Drying Method Using Vegetable Oil

ABSTRACT

Healthcare waste must be managed properly due to the hazards they impose on public health and the environment. In this study, an alternative method of treating infectious waste via thermal treatment using coconut oil was investigated in a laboratory-scale setup. The effects of oil temperature (121°C, 145°C and 170°C) and treatment time (10, 20 and 30 minutes) on bacterial growth and properties of simulated infectious wastes contaminated with Bacillus subtilis were determined. No bacterial growth was observed in the samples even at the seventh day after treatment using 145°C (20- and 30-minute treatment time) and 170°C (all treatment time). However, growth on enriched media occurred for the samples treated at 170°C, indicating possible spore germination. The treatment at 145°C and 30 minutes was effective in treating contaminated syringes and cloths. The contaminated meat samples underwent thermal degradation and had a maximum weight reduction of 74.1%, which was mainly due to moisture loss. The cloths, however, did not change in its appearance but syringes and other plastics melted and deformed. Thus, the thermal treatment was found to be a good disinfection method, causing severe damage to cells. The treated infectious waste materials can be disposed in landfills without potential recurrence of bacterial growth.

Key words: *Infectious waste, thermal treatment, coconut oil, Bacillus subtilis*

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INTRODUCTION

Facilities dedicated to the treatment of patients such as hospitals, clinics, medical posts, analytical laboratories, and dialysis facilities, among others, generate health care wastes, which refer to all materials, biological or non-biological, that are discarded from such facilities and are not intended for any other use. Among the different kinds of healthcare wastes, infectious wastes are an urgent concern since they can potentially transmit infectious diseases. Infectious wastes may contain infected "sharps" and pathological wastes which consist of blood, body fluids, tissue, organs, body parts, human fetuses, and animal carcasses (*Diaz and Savage 2003*). Improper handling and disposal of these wastes can affect healthcare workers, waste handlers, patients and visitors in the health care facility, landfill operators, scavengers, and the public, in general (*Department of Health 2004*).

According to a report by the Asian Development Bank in 2003 (*Diaz and Savage 2003*) the total waste production in hospitals in Metropolitan Manila, Philippines, was about 0.54 kg d^{-1} per bed, which consisted of infectious wastes generated at 0.34 kg d^{-1} per bed (63%) and non-infectious wastes generated at 0.20 kg d^{-1} per bed (37%). In addition, the percentage of total health care waste of the total amount of municipal solid waste generated isin the order of 0.72% while the percentage of infectious waste of the municipal solid waste generated is about 0.27%. Thus, proper management of infectious wastes must be implemented. However, in a case study report on hospital waste management in Metro Manila, *Soncuya, Matias and Lapid* (*1997*) obtained the following findings: there were no satisfactory and environmental friendly disposal facilities for hazardous hospital wastes; the existing treatment capacity was insufficient for the proper disposal of the major part of medical wastes generated in Metro Manila area; pre-treatment of infectious waste was not practiced; and that due to inadequate segregation of infectious from non-infectious wastes, much of the generated hospital waste was disposed directly to municipal landfills, some of which were uncontrolled and occupied by thousands of squatters.

Nevertheless, the *Department of Health* (*2004*) listed various technologies that can be applied for health care waste treatment, and these include: thermal processes such as pyrolysis, microwave and autoclave, chemical disinfection, biological processes, radiation technology, encapsulation and inertization. Incineration of waste has been banned since the implementation of the Republic Act 8749, otherwise known as the "Philippine Clean Air Act of 1999." Thus, the autoclave technology became the most commonly used for healthcare waste treatment. Currently, there are six authorized healthcare waste treaters in the country, using four different health care waste treatment technologies (*Department of Health 2004*): autoclaves, hydroclave, microwave technology (*Benedictos 2011*) and

pyrolysis system. Except for the pyrolysis system, all the other technologies are limited in handling pathological wastes (**Table 1**). In addition, waste volume reduction cannot be attained by the treatments alone but rather require addition of mechanical equipment such as shredders and grinders. Waste volume reduction is necessary for easier handling of treated waste during disposal.

Based from the issues presented regarding infectious waste treatment and management, there is a need to investigate alternative technologies for infectious waste treatment. One such technology is by thermal treatment using oil. Thermal treatment by frying was employed in the drying of waste sludge by *Romdhana et al.* in *2009* for the reduction of pathogen population in the sludge. The process involved immersing the material in a heated non-miscible fluid (animal fat, waste oil, etc.) at a temperature range of 110°C to 180°C under atmospheric conditions (*Peregrina et al. 2006*). Pathogen concentration of 7 x 10^4 CFU/100 mL initially present in the sewage sludge was significantly reduced and the sewage sludge was completely disinfected when heated for 10 min during the fry-drying process. Several other studies investigated thermal treatment by frying or hot oil immersion of sludge, but these studies focused more on moisture reduction to render the suitability of the treated sludge as an energy source (*Ohm et al. 2010*).

To the best knowledge of the authors, no study has been made regarding the thermal treatment of infectious wastes using oil as the heating medium. The minimal complexity of this process, as well as its capacity to accommodate pathological wastes, makes it a potential treatment option for infectious waste disinfection. Thus, this study was conducted to evaluate the treatability of simulated infectious waste by thermal treatment using coconut oil as the heating medium. Infectious waste was simulated by contaminating ground meat with *Bacillus subtilis*, which is one of the many test or indicator organisms that can be used to evaluate medical waste treatment technologies. The spores of *B. subtilis* are resistant to thermal disinfection and it can be used as a surrogate pathogen because it exhibits a thermal death rate similar to Clostridium species, a pathogenic spore-forming bacterium, which is considered as one of the most heat resistant human pathogens (*Cole et al. 1991*). As such, the specific objectives of this study were to: determine the effect of oil temperature and treatment time on bacterial growth in the simulated infectious waste after treatment; determine the suitability of the treated waste for pathogenic recurrence and the time required before the recurrence of pathogens; determine the reusability of the oil after treatment; and evaluate the weight reduction of the waste and its appearance or recognizability before and after treatment.

MATERIALS AND METHODS

Synthetic Hosts Sterilization

Ground raw (pork) meat, plastic syringes with needles and cloths/bandages were used as bacterial hosts to simulate the infectious waste. The raw meat was divided into small portions of about 65 g per sample, which were wrapped in aluminum foils. These synthetic hosts were sterilized in an autoclave at 121˚C and 15 psig for 1h. This is to eliminate the presence of microorganisms other than the test bacteria. This also allows the control on the initial concentration of the test bacteria in the hosts.

Preparation of *Bacillus subtilis* pure culture

A pure culture of *Bacillus subtilis* was obtained from the National Institute of Molecular Biology and Biotechnology (BIOTECH), UPLB. It was propagated in nutrient agar slants for series of experiments. In each batch experiment, a loop of bacteria was transferred to a 200-mL nutrient broth for a 24-hr aerobic growth. A standard curve relating the bacteria concentration and optical density was established using spectrophotometer and plating method. Hence, the initial concentration of bacteria in the suspension could be adjusted to the desired concentration by dilution. When the density was about 1x10^8 CFU mL^{-1}, 1mL of the suspension was injected to each synthetic host.

Disinfection by Fry-Drying Method

Four liters of fresh commercial coconut oil was poured into the pan, as illustrated in **Figure 1**. The oil was heated up to the set temperatures while the thermocouple was immersed in the oil to monitor the oil temperature. When the set temperature was attained, it was maintained for about 1 min. The sterilized hosts were contaminated with bacteria of about 1x10^8 CFU mL^{-1} concentration. The contaminated hosts were placed into the basket and then submerged in the heated oil. The timer was started to measure the treatment time. The cover was used to prevent splattering during treatment. Samples were taken every 10 minutes and stored in sealed sterile plastic containers at room temperature. Each condition treated 8 contaminated hosts, which will be taken daily for bacterial cell count. One treated sample was taken right after the treatment and labeled “Day 0” for that specific treatment condition. Samples were taken every day from Day 1 to Day 7. After a week, another sample was taken and labeled Day 14. These samples were dispersed in liquid media and analyzed for colony counts using drop-plate technique.

The main factors that were varied in the experiments were the oil temperature (T) and treatment time (t). The temperature settings were varied at 121˚C, 145˚C and 170˚C

Table 1. Comparison of various health care waste treatment technologies (Department of Health, 2004).

Features	Autoclave	Microwave	Pyrolysis Systems	Chemical Treatment
Treatment Method	Steam treatment	Microwave generator frequency	Pyrolysis chamber at elevated temperatures	Chemical (sodium hypochlorite) and mechanical (hammer mill)
Post-treatment Procedure	Shredding after disinfection	Shredding and granulation	Boxed	Shredding and grinding
Operating Temperature	120-160^{o}C	110^{o}C max	Plasma pyrolysis: 1650^{o}C Laser-based pyrolysis: 3,300^{o}C - 5,500 ^{o}C	None
Energy	Boiler	75 kW	300 kW	400 Volts
Capacity	100-1,500 lbs hr^{-1}	230-900 lbs hr^{-1}	Plasma pyrolysis: 1-10 t day^{-1} Laser-based pyrolysis: 5-100 t day^{-1}	30-3,000 lbs hr^{-1}
Recognizable Waste	Yes	Yes	Yes	Yes
Waste Volume % Reduction	80-85% with shredder, mass essentially the same	80-85% with shredder, mass essentially the same	80-95% reduction both in mass and in volume depending on the waste matrix	60-90% with shredding and heat treatment
Residues	Inert waste	Inert waste	Inert slag and combustible gases	Chemical wastes for treatment
Environmental Pollutants	Possible odors	Odor problem in immediate vicinity	No liquid effluent	Very high noise levels, offensive odor, liquid effluents
Environmental Control	Closed system	Closed system with HEPA filters	Scrubber	HEPA filters
Advantage	Waste suitable for landfill; well-proven technology	Waste suitable for landfill; well-proven technology	Waste suitable for landfill; recovers at least 80% heat in the form of hot water steam	Well-proven technology
Disadvantage	Possible odors	Possible odors; large metal object can damage the system	Relatively high capital cost; maybe extensive maintenance cost. May produce dioxins and furans at very small quantities	Chemical management and disposal required because of use of chemical agents
Limitations	No body parts, chemical and pharmacological wastes; however, advanced autoclaves can treat anatomical parts	No body parts, liquid blood and hazardous chemical substances	No lead, cadmium, and mercury	No human and animal remains, chemical wastes

while the treatment times included 10 minutes, 20 minutes and 30 minutes. Duplicate runs were conducted and additional trials were done as controls: positive and negative controls. The negative runs determined whether contamination or addition of bacteria from any outside source happened during the treatment or not. On the other hand, the positive control was prepared by addition of known concentration of bacteria after the treatment. This accounted for the accuracy of the plating technique and analysis. Full factorial design was used to analyze the treatment data relating the effects of oil temperature and treatment time to the number of days to grow the cells in the treated hosts.

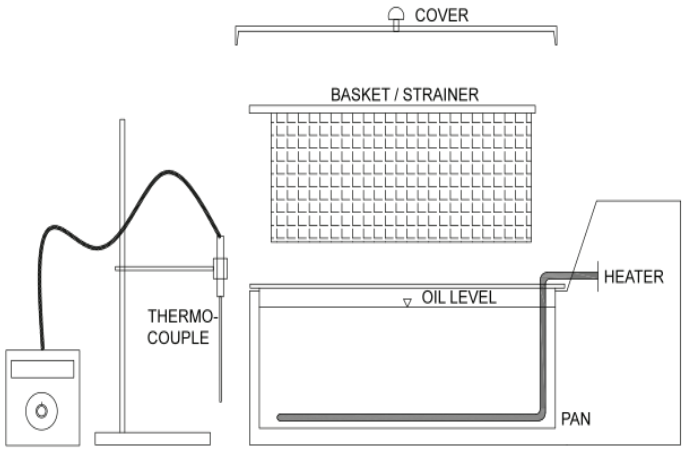

Figure 1. Thermal treatment setup using oil as treatment medium.

Recurrence Test in Enriched Media

It was assumed that at 170˚C, the bacterial cells were severely damaged at all treatment times. However, there is a possibility that these cells could still recover when grown on a favorable environment with enough time for the cells to recover. Therefore, samples were taken from the materials treated at 170˚C for 10, 20 and 30 minutes of treatment time. The samples were soaked and the cells were allowed to disperse in a nutrient broth to deliberately grow the cells that survived the treatment. After 24-hour of shaking and incubation, a sample from the broth was analyzed for bacterial cell count using optical density.

Sample Preparation and Analysis

After each treatment, the cells, both active and injured, were dispersed in solutions to be recovered and eventually grown with or without nutrients to simulate different environments. Thus, samples were dispersed in 85% saline solution and shaken for 15 minutes and were analyzed using the modified Miles and Misra drop-plate technique. This cell count technique has a minimum detection limit of 100 CFU mL^{-1} (*Honeyborne et al. 2011, Miles and Misra 1938*). The preliminary experiments showed that the lowest count of colony units was 2 x 10^2 CFU mL^{-1}. A sample from the dispersing solution was taken to undergo serial dilution up to 10^3. Each dilution was poured onto an agar plate and incubated for 10-12 hours to grow the cells on the plates. The number of colonies was counted and recorded. This was done right after the treatment (Day 0) and then daily until the seventh day (Day 7). The same technique was done for Day 14 samples.

Reusability Test

From the factorial runs, the best condition was determined, adopted and used in the reusability experiments. These experiments were done by using the same oil for four (4) treatment batches. The disinfection effectiveness of the oil was described in every batch by determining the recurrence of the cells.

RESULTS AND DISCUSSION

Effect of Oil Temperature and Time on Viable Cell Growth

Analysis of the samples right after treatment (i.e. Day 0) shows a cell count less than the detection limit for all treatment times and temperatures, indicating that most of the cells were damaged after the heat treatment (**Table 2**). This means that disinfection using thermal treatment with oil is an effective technique even at the minimum temperature level (121˚C) and shortest treatment time (10 minutes). Disinfection is the elimination or inactivation of most or all of pathogenic microorganisms except the bacterial spores (*Rutala and Weber 2008*).

However, cell growth occurred one day after the treatment for the 10-minute treatment time and temperatures of 121˚C and 145 ˚C, and after two days (Day 2) for both 20 min and 30 min treatment times at 121˚C. This indicates that the viable cells were able to recuperate from the detrimental effect of the heat treatment. The cells of *B. subtilis* are Gram-positive, rod-shaped, and grows in the mesophilic temperature range with optimal range of 25-35˚C. When exposed to different hostile, stressful and starved conditions, *B. subtilis* cells respond by forming stress-resistant spores and production of stress proteins. Bacterial endospores are the most resistant form of microbial life and are liberated by lysis of the parent vegetative cell when nutrients are depleted after the exponential phase of growth, or when the temperature, pressure, or moisture reached values adverse for cellular life (*Didomenico 1992*). The presence of various layers of spore coat, cell membrane and spore membrane serves as a protection towards the hostile conditions (*Setlow 2006*). In this study, the presence of growth of *B. subtilis* cells one or several days after the thermal treatment indicates that the conditions became favorable again for vegetative cells to form from the endospores. The treated samples for the 121˚C and 30 minute treatment combination after two days were still recognizable as meat and contain components that can still support bacterial growth (**Figure 2**). Therefore, the samples treated at these conditions must be transferred to the special and secured cell in landfills within one or two days, where the conditions would not be able to support cell growth.

For the treatment at 145˚C and 20 and 30 minutes treatment times, as well as at 170˚C for all treatment times, the number of colonies was below the detection limit even at Day 7 after treatment. The bacterial cells were damaged or

Table 2. Bacterial growth in the test materials after thermal treatment.

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
121˚C	10 mins	<DL	+	+	+	+	+	+	+
	20 mins	<DL	<DL	+	+	+	+	+	+
	30 mins	<DL	<DL	+	+	+	+	+	+
	(-) control	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	(+) control	+	+	+	+	+	+	+	+
145˚C	10 mins	<DL	+	+	+	+	+	+	+
	20 mins	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	30 mins	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	(-) control	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	(+) control	+	+	+	+	+	+	+	+
170˚C	10 mins	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	20 mins	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	30 mins	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	(-) control	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	(+) control	+	+	+	+	+	+	+	+

Notes: 1. (+), Positive number of cell counts indicating growth of bacterial cells;
2. DL is lower detection limit (100 CFU mL^{-1}); and
3. Test materials were suspended in saline solution.

destroyed and the meat after the treatment was not also conducive for cell growth. The treated materials two days after treatment are dried and toasted with no apparent microbial activity observed (e.g. emission of odor) (**Figure 2 b** and **c**).

An important factor for cell growth that was removed by the thermal treatment is moisture or water content, which is necessary for assimilation of the cells. All bacteria require water for growth and reproduction. Low water level results to longer lag phase and slower growth, impaired transport, and loss of membrane fluidity. Most nutrients are water-soluble. Water is necessary to transport the nutrients across the cell membrane (*Willey et al. 2009*). In effect, after removal of water from the sample, bacterial growth could not be facilitated (**Figure 2 b** and **c**).

For comparative purposes, the number of cells in the treated samples was also determined theoretically using equations obtained from the concept of cell death kinetics during thermal destruction of the bacterial cells. According to *Najafpour* (*2007*), the thermal treatment or destruction of microorganisms follows a first-order chemical reaction rate model which can be simplified further to:

$$\ln\frac{N}{N_o} = -k_o\, e^{\left(-\frac{E}{RT}\right)} t \qquad \text{(Equation 1)}$$

where: N = number of viable organisms present
t = treatment time
k_o = pre-exponential factor
E = activation energy
R = gas constant
T = absolute temperature

Specifically for *B. subtilis*, the values of activation energy, E, and pre-exponential factor, k_o, are 68.7 kcal mol^{-1} and 9.5 x 10^{37} $minute^{-1}$, respectively (*Jackson 1990*). The initial cell concentration, which was previously determined, was $1x10^8$ cells per sample.

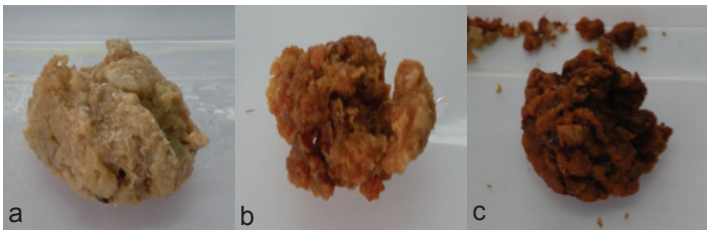

Figure 2. Two-day old samples treated for 30 minutes at (a) 121˚C, (b) 145˚C and (c) 170˚C.

Only the treatments at 121˚C for all treatment times yielded values showing the presence of bacterial growth (**Table 3**). For 10 minutes, the treatment efficiency is 99.93%. These surviving bacterial cells were responsible in the cell growth observed one day after the treatment. The cells might be injured and needed 24 hours to recover. The same might have happened to the 20 minutes and 30 minutes of treatment at 121˚C but the surviving cells after these treatment conditions required two days to recover. The treated meat offered a suitable environment for the recovery, as previously discussed.

The actual cell count, however, after the treatment at 145˚C for 10 minutes did not conform to the theoretical cell count, which is nil. The difference might be attributed to the inefficient treatment due to unmaintained oil temperature and heat losses during the duration of the process at 10 minutes. When the contaminated meat samples were loaded, the set temperature, which was initially at 145˚C, decreased and remained lower than the set point for 1-2 minutes. This might be considered as a warm-up period in the treatment where the addition of the contaminated samples destabilizes

Table 3. Theoretical final cell counts after treatment at different oil temperature and treatment time.

Temp (T), ˚C	Time (t), minutes	Final Cell Count (N), cells/sample
121	10	70900
121	20	50.30 (<DL)
121	30	0.034 (<DL)
145	10	0 (<DL)
145	20	0 (<DL)
145	30	0 (<DL)
170	10	0 (<DL)
170	20	0 (<DL)
170	30	0 (<DL)

Note: DL is lower detection limit (100 CFU mL^{-1})

the oil temperature until the oil reaches back the set treatment temperature. Thus, the duration of the treatment at the desired temperature was actually less than 10 minutes. With respect to the longer treatment times, the effect of the two-minute warm-up time was more pronounced in the 10-minute treatment than in the 20-minute and 30-minute treatment times.

Theoretically, no bacterial growth will be obtained for the treatment at 170˚C, even at the shortest heating time, which agrees with the experimental results. In this case, the effect of temperature overruled the effect of time on the treatment efficiency, such that the temperature was high enough to kill the bacterial cells for all treatment times. The treated material at this temperature was not favorable for bacterial cell growth.

Recurrence in Enriched Media

When the samples were treated at 170˚C, the treated hosts were considered not conducive for damaged cell's recovery and growth. Thus, the treated samples were dispersed in nutrient broth to facilitate the cell recovery. The cell concentrations in the test materials treated for 10 minutes, 20 minutes, and 30 minutes were then measured to be TNTC (too numerous to count), 2.20 x 10^5 cells mL^{-1}, and 7.85 x 10^5 cells mL^{-1}, respectively. This means that there were injured cells that remained in the materials after treatment that were not able to recover unless subjected to a favorable environment. This also suggests that the spores are still viable but could be just severely damaged that they were not able to germinate properly and grow in the saline solution and agar media.

Aside from the experimental results, the initial cell count of the treated sample prior to growth on enriched media was calculated theoretically using the equation for the doubling time. Doubling time occurs during the exponential phase when the cell is dividing at constant time intervals. Since the population doubles every generation, the increase in cell count is always 2^n where n is the number of generations (*Willey et al. 2009*). Therefore, the cell count after time *t* can be calculated as follows:

$$N_t = N_o \times 2^n \qquad \text{(Equation 2)}$$

where: N_t = cell count at time *t*
N_o = initial cell count
n = number of generations in time *t*

The number of generations *n* can be calculated from the product of the mean growth rate constant *k* and the time *t*. The mean growth rate constant *k* can be calculated from the doubling time (g) for *B. subtilis*, which is 0.43 hour (*Willey et al. 2009*):

$$k = \frac{1}{g} = \frac{1}{0.43} = 2.3256 \frac{generations}{hour} \qquad \text{(Equation 3)}$$

Thus, for 24 hours of incubation, the number of generations (n) was 55.81 generations. Taking into account the liquid media volume of 200 mL and the mass of the treated sample of about 20 g, the initial cell counts (N_o) of the samples from 20min and 30min treatment at 170˚C are 2.48 x 10^{-11} CFU mL^{-1} and 6.97 x 10^{-10} CFU mL^{-1}, respectively. These values were far below 1 viable cell, which indicates that the cells that multiplied in 24 h took long hours of recovery before they replicated. This indicates that the growth of the cells was deterred because of injury. The damage inflicted on the cells by the heat treatment must be great to cause longer time of recovery despite good conditions for bacterial growth.

In actual and real environment, landfills are not suitable for the growth of pathogenic microorganisms. Human pathogens are very specialized microorganisms that need specific conditions to survive and grow. The conditions in landfills are extremely different than those in the human body. The temperature, nutrients and oxygen availability, as well as competition with other microorganisms are constantly varying through time that makes it an unfavorable site for pathogen growth. Obligate human pathogens cannot live in landfill site and do not have the capability to adapt to landfill conditions (*Katoch 2008*). If the cells were severely damaged, recovery and multiplication are not possible in landfills. Thus, the treated samples that exhibited no signs of bacterial growth can be disposed in landfills.

Effect of Thermal Treatment on Oil Quality and Reusability

The treatment oils were also analyzed to determine whether the damaged bacterial cells left in the oils recovered and multiplied. For the oil used at 121˚C, the cells might not be severely damaged and was able to replicate in 2 days

Table 4. Bacterial growth in the oils after thermal treatment.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
121°C	<DL	<DL	+	+	+	+	+	+
145°C	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
170°C	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Notes: 1. (+), Positive number of cell counts indicating growth of bacterial cells;
2. DL is lower detection limit (100 CFU mL^{-1}); and
3. Test materials were suspended in saline solution.

(**Table 4**). In the oils used at 145˚C and 170˚C, the cells that were retained in the oil were not able to recover due to injury inflicted by the treatment. These results conformed to the quality of damage done to the cells in the test materials.

In the determination of reusability of oil, the maximum temperature level (170˚C) was used for 30 minutes. This was used since the temperature is near the smoke point that gives a higher degree of oil degradation. The oil was used four times consecutively in treating four separate batches of contaminated meat samples. The treated samples were analyzed right after the treatment (Day 0), after one day (Day 1), after seven days (Day 7), and after 14 days (Day 14).

The oil was still effective in the thermal treatment of the fourth batch of samples since the cells were damaged and were not able to grow even after Day 14 (**Table 5**). Although the treatment performance of the oil was maintained even after several reuse, the color darkened after each batch of treatment. Also, by ocular inspection, the oil changed its color from yellowish to reddish and contained solid residues after the last batch of treatment. This should not be a problem since the treatment depends on the operating temperature and not on the physical appearance of the oil. As long as the oil is capable of reaching the set temperature, the oil can perform the treatment although the solids that are suspended must be removed by screening so that these solids do not hinder the transfer of heat from the oil to the test material. However, oil loss may become significant with continuous reuse and thus, will require oil supplementation if to be used further. Treatment using mixtures of fresh and reused oil was not further explored in this study since there was no observed significant oil loss after the fourth batch of treatment. This positive result in the reusability of the oil translates to an economic advantage of this type of thermal treatment of infectious wastes.

Table 5. Number of days for growth of damaged bacterial cells in four batches of test materials treated with the same oil.

Batch	Number of days required for bacterial cell growth
1	>14
2	>14
3	>14
4	>14

Notes: 1. Four consecutive fresh batches of infected ground meat were treated at 170˚C for 30 minutes.
2. Test materials were suspended in saline solution.

Two-Factor Interaction Response Model

A model equation was developed relating the oil temperature and time to the number of days it would take for bacterial growth to occur in the treated samples. The value used for the maximum number of days without cell growth was 14 days. After the seventh day of analysis, the samples were kept in their respective containers until the fourteenth day to determine whether cell growth will still occur.

The coded form of the factors used in the experiments where (+), (-) and Y, which are described as the high levels, low levels and the response variable, i.e. the number of days for cell growth (**Table 6**). Factor A is the temperature with high and low levels of 170˚C and 121˚C, respectively while factor B is the treatment time with high and low levels of 30 min and 10 min, respectively. It was found that the number of colony units after at least 14 days of storage was still below detection limit.

By statistical calculations, the model for the obtained results was found to be significant at 95% confidence interval. The individual and interaction effects of independent variables significantly affect the response and the relationships are described by the following equation:

$$Y = 7.75 + 6.25A + 0.25B - 0.25AB \quad \text{(Equation 4)}$$

Based on Equation 4, increasing the individual factors results to increase in the mean number of days (i.e. 7.75 days) necessary for cell recovery and growth. The equation suggests that the oil temperature (in ˚C) causes greater increase in degree of cell damage than treatment time (in minute). In disinfection, the greater the damage, the longer it takes for the cells to recover and grow. The interaction of the factors (AB), however, provides a slight negative effect to

Table 6. Coded table of factors and responses.

Runs	A	B	Y_{ave}
1	+	+	>14
2	+	-	>14
3	-	+	2
4	-	-	1

the response. This corresponds to the typical heat treatment methods, where increasing the treatment temperature entails shorter treatment time for the same disinfection efficiency.

It can be seen that there are deviations in terms of actual values of response from the model. Nevertheless, the deviation of the line (slope = 0.8323) to unity still suggests that the model can approximate the response based on varying parameters (**Figure 3**).With the relationship between the oil temperature and the number of days for bacterial cell growth, the best treatment options are the maximum levels of temperature and time used in the experiment, i.e. 170˚C for 30 min. However, it is practical to operate at a lower temperature, and based on the plot, the treatment at 145˚C gave almost the same results as the treatment done at 170˚C except with 10 min treatment time (**Figure 4**).

Disinfection of Contaminated Syringes and Cloths

The oil temperature and treatment time used to treat contaminated syringes and cloths were 145˚C and 30min, respectively. This suggests that the treated host materials did not provide a favorable environment for cell growth, which is expected since these are mainly polymeric materials and cannot provide the nutrients needed for cell growth (**Table 7**).

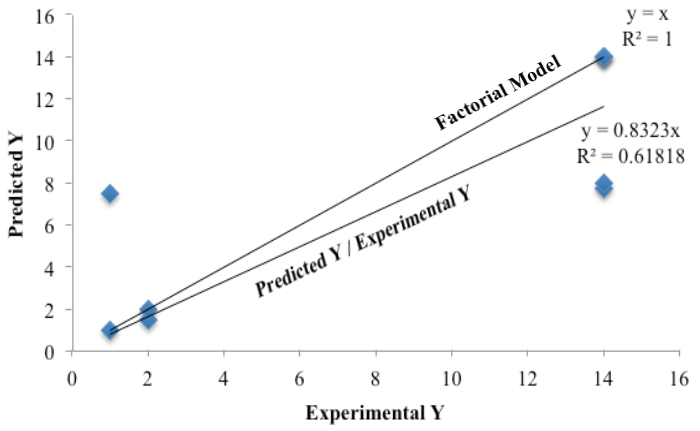

Figure 3. Deviation of the actual response from the predicted response based on the factorial model.

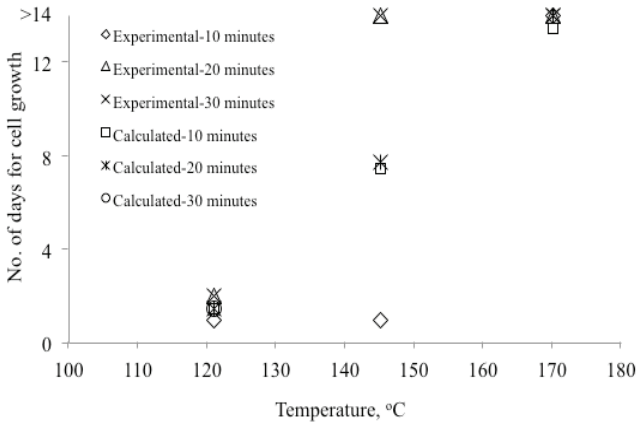

Figure 4. Plot of the number of days for cell growth versus the oil temperature using experimental and calculated data.

Physical Characteristics of the Treated Materials for Waste Handling

Aside from ensuring that no bacterial growth can occur in the treated materials, it is also important to assess the recognizability and appearance of the wastes since they will still be disposed in landfills after treatment. Also, the handling and transportation of the treated wastes will depend upon their physical appearance. The samples treated at the lowest temperature shows "rawness" and presence of moisture. The samples after treatment at the higher temperatures were dry and tend to form crumbs (**Figure 2**). The weight reduction in the samples treated at 145˚C and 170˚C were determined and are shown in **Table 8**. The reduction in weight of the samples can be attributed to the evaporation of water from the samples. According to the *Food and Agriculture Organization* (*2007*), lean pork has 75.1% water, 22.8% protein, 1.2% fat, and 1.0% ash. Among the components of the meat, the water has the highest tendency to be removed.

During thermal treatment, splattering of the oil became vigorous when the samples were loaded since the water coming from the samples turned into superheated steam instantaneously. The splattering continued until most of the water escaped the treatment chamber. The meat samples tend to shrink and crumple when heated until they become dried and resulted in the easy handling of the waste. In effect, the loss of water made the material brittle and very easy for breakage and size reduction. This is advantageous since greater amount of the treated samples can be accommodated easily during handling and transportation to landfills.

On the other hand, there were no significant changes in the physical characteristics of syringes and cloths. The plastic materials melted and were deformed after the treatment while the bandages absorbed oil which needs to be pressed out since it may increase the weight of the samples and could become a source of spillage during handling.

CONCLUSION AND RECOMMENDATIONS

This study explored the effectiveness of simulated infectious waste disinfection by thermal treatment using coconut oil. The test materials used in the experiments (ground meat, syringes and bandages/cloths) were contaminated with *Bacillus subtilis*, which has high resistance to thermal treatment. The contaminated materials were treated with coconut oil heated at different temperatures: 121˚C, 145˚C and 170˚C while the duration of treatment was varied at 10 min, 20 min and 30 min. The treated samples were soaked

Table 7. Bacterial growth in syringe and cloth after thermal treatment.

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Syringe	145˚C	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cloth	145˚C	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Notes: 1. (+), Positive number of cell counts indicating growth of bacterial cells;
2. DL is lower detection limit (100 CFU mL^{-2}); and
3. Test materials were suspended in saline solution.

Table 8. Weight reduction in the meat samples after treatment.

Temperature, ˚C	Ave initial weight, g	Ave final weight, g	% weight reduction
145	65.455	24.34	62.81
170	62.305	16.145	74.09

Note: The meat samples were treated for 30 minutes.

in saline solution to disperse the viable cells. A portion from the saline solution was analyzed for cell count using Miles and Misra plating method. The test materials were also analyzed for its physical characteristics such as color and weight reduction.

All temperature-time combinations were effective in disinfecting the contaminated materials since most bacterial cells were severely damaged based from the analysis of the samples right after treatment. However, treated meat samples at 121˚C were still conducive for bacterial growth one or two days after the treatment, which suggests that the cells were barely damage. However, in the case of oil temperatures 145˚C and 170˚C, the colony counts were below detection limit at the 14th day after treatment except for the 145˚C-10min operation. When the treated samples at 170˚C were dispersed in nutrient broth and incubated for 24h, growth occurred suggesting that the cells were not completely destroyed but required more favorable environment for growth. The damage was severe that the recovery took a long time before the cells reproduced despite a very suitable condition for bacterial growth.

The oil temperature affected the number of days for bacterial growth recurrence more than the treatment time, as indicated in the model equation. The linear relationship between the temperature and time and the number of days for bacterial growth suggests that bacterial growth is hindered at higher oil temperature and time. With this, the best conditions should be the maximum (170˚C-30 minutes), but the treatment at 145˚C also provided acceptable results, making it a better treatment temperature option for economical reasons.

In terms of physical characteristics, the waste materials were not recognizable after treatment due to color change, water content reduction (74%) and thermal degradation. Since water is an essential requirement in bacterial growth, drying played a significant role in the disinfection process. Thus, disinfection of simulated infectious wastes by thermal treatment using oil is effective in preventing bacterial growth and the chance of bacterial recurrence if disposed to unsuitable environment for human pathogen growth such as landfill.

For some recommendations, further studies may be performed using smaller temperature intervals above 121˚C to around 145˚C to determine the minimum temperature at which bacterial recurrence will not occur. The measurement and the effects of different levels of rancidity on the treatment efficiency may be an area for further study. Furthermore, an actual used coconut oil from quick-serve restaurant may be considered as medium for treatment.

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