Modeling of pathogen inactivation in thermal septic tanks

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ABSTRACT

Thermal application has been widely used for pathogen inactivation in various fields. The purpose of this research was to develop a model of pathogen inactivation in septic tanks operating at various temperatures. Four laboratory-scale septic tanks fed with septage were operated at temperatures of 30, 40, 50 and 60 °C and *Escherichia coli* (*E. coli*) was selected as the pathogenic indicator. The efficiencies of *E. coli* inactivation were found to increase with increasing temperatures, while the opposites were observed for chemical oxygen demand (COD) reduction. At 60 °C, the *E. coli* concentrations were reduced from 9.6×10^6 to about 10 most probable number (MPN)/100 mL or 6 log reduction. The kinetics of *E. coli* reduction followed a modified Weibull model which could be applied to septic tank design and operation. The percentage COD removal was found to be 93, 94, 89 and 84 at temperatures of 32, 40, 50 and 60 °C, respectively. The results of this study suggested that pathogenic microorganisms in septic tanks could be inactivated to be at a safe level with thermal application.

Key words | *E. coli*, methanogens, modified Weibull model, pathogen inactivation, septic tanks, temperatures

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ABBREVIATIONS

b_T	temperature-dependent coefficient, d^{-1}
BOD_5	5-day biochemical oxygen demand
COD	chemical oxygen demand
d	day
h	hour
k_T	first-order rate constant at temperatures
L	liter
N_t	number of *E. coli* at time *t* (MPN/100 mL)
N_0	initial number of *E. coli* (MPN/100 mL)
N	Weibull coefficient
t	retention time, d
T	temperature, °C
TKN	total Kjeldahl nitrogen
TS	total solids
VSS	volatile suspended solids
γ	regression factor

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INTRODUCTION

At present more than 2 billion people worldwide still lack access to improved sanitation and more than 15 million people, especially from developing countries, die every year from infectious and parasitic diseases as a result of poor sanitation (WHO 2013). Centralized wastewater treatment systems, practised in most developed countries, do not seem appropriate for developing countries because of high investment and operation costs and unavailability of skilled manpower. Septic tanks are the most commonly used onsite sanitation technologies to treat household wastewaters in areas without centralized sewer systems (Rybczynski *et al.* 1982). However, septic tanks constructed in most developing countries usually do not have leaching fields or drainage fields to further treat septic tank effluent. Instead, these septic tank effluents are allowed to seep into surrounding soils or are directly discharged into nearby storm sewers or water courses. Owing to the short retention time (about 1–3 days) in the septic tanks, the septic tank effluent is still very polluted, containing high concentrations of organic matter, nutrients and pathogenic microorganisms (Leclerc *et al.* 1977; Eriksson *et al.* 2002). Thus, these septic tank effluents can contaminate nearby surface and ground water resources or soil, causing water pollution and health risks to the public.

Since septic tanks will continue to be an onsite sanitation technology in developing countries, methods to improve septic tank performance to alleviate the above problems should be considered. It is a well-known fact that temperature could enhance pathogen die-offs (Carrington 2001; Winblad & Simpson-Hébert 2004) and increase microbial activity or biodegradation of organic matter (Metcalf & Eddy 2003). Therefore, operating a septic tank at temperatures higher than ambient conditions should theoretically produce better quality septic tank effluent. Thermal treatment of wastewater sludge and raw sewage was previously conducted by Mocé-Llivina *et al.* (2003). Their results revealed that *Escherichia coli* numbers in the sludge and raw sewage samples were reduced by about 3.6–6 log within 30 minutes of heat exposure at 60–80 °C. Pandey & Soupir (2011) found that anaerobic digesters treating dairy manure offered the highest *E. coli* inactivation at 52.5 °C when compared to the results obtained at 37 and 25 °C.

To date, there have been very few reports on thermal application to septic tanks, only to thermophilic anaerobic digesters. The overall objective of this study was to investigate the effect of temperatures on pathogen inactivation and to develop a model to describe the pathogen inactivation efficiency. The specific objectives are listed below:

1. To evaluate the efficiencies of pathogen reduction in laboratory-scale septic tanks operating at temperatures varying from 30 to 60 °C.
2. To determine an appropriate model for pathogen inactivation in septic tanks.
3. To observe the effects of temperature variation in septic tanks on the efficiency of COD reduction and biogas production.
4. To validate the developed inactivation model with laboratory-scale and actual septic tanks.

MATERIALS AND METHODS

This study employed laboratory-scale septic tanks and septage samples collected from household communities in central Thailand. *E. coli* bacteria were used as a pathogenic indicator.

Operation of laboratory-scale septic tanks

Four laboratory-scale septic tanks, each with a dimension of 64 cm × 25 cm × 40 cm (length × width × depth) or a working volume of 40 L, were constructed with clear acrylic plastic. Temperatures of these septic tanks were maintained at ambient (30–32 °C), 40, 50 and 60 °C using heated water circulating around the reactors (Figure 1(a) and 1(b)). To minimize heat loss, each septic tank was insulated with aluminum foil. An on-line temperature sensor (Thermocouple type K (CA) [series SK PCR-1)] and a gas collection chamber were installed in each reactor. To start up the operation, each reactor was fed with 40 L of septage sample collected from a household community and the anaerobic bacteria were allowed to acclimatize until biogas generation was observed.

After acclimatization, the effluent samples were collected for analysis of pH, chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), volatile suspended solids (VSS), total solids (TS), total Kjeldahl nitrogen (TKN) and *E. coli* concentrations. Biogas samples were collected and analyzed for the volume and percentage content of CH_4.

Experimental study on kinetics of *E. coli* inactivation

To determine the effects of temperatures on *E. coli* inactivation, each of the 50 mL glass bottles was fed with about 10 mL of septic tank sludge and 20 mL of septic tank effluent collected from a household in central Thailand. To simulate conditions occurring in actual septic tanks which normally have no mixing, only intermittent flows, these glass bottles were thoroughly mixed at the beginning and incubated under anaerobic conditions (by purging with N_2 gas) in a water-bath and maintained at temperatures of 32, 40, 50, 60 and 70 °C. After 1, 3, 6,

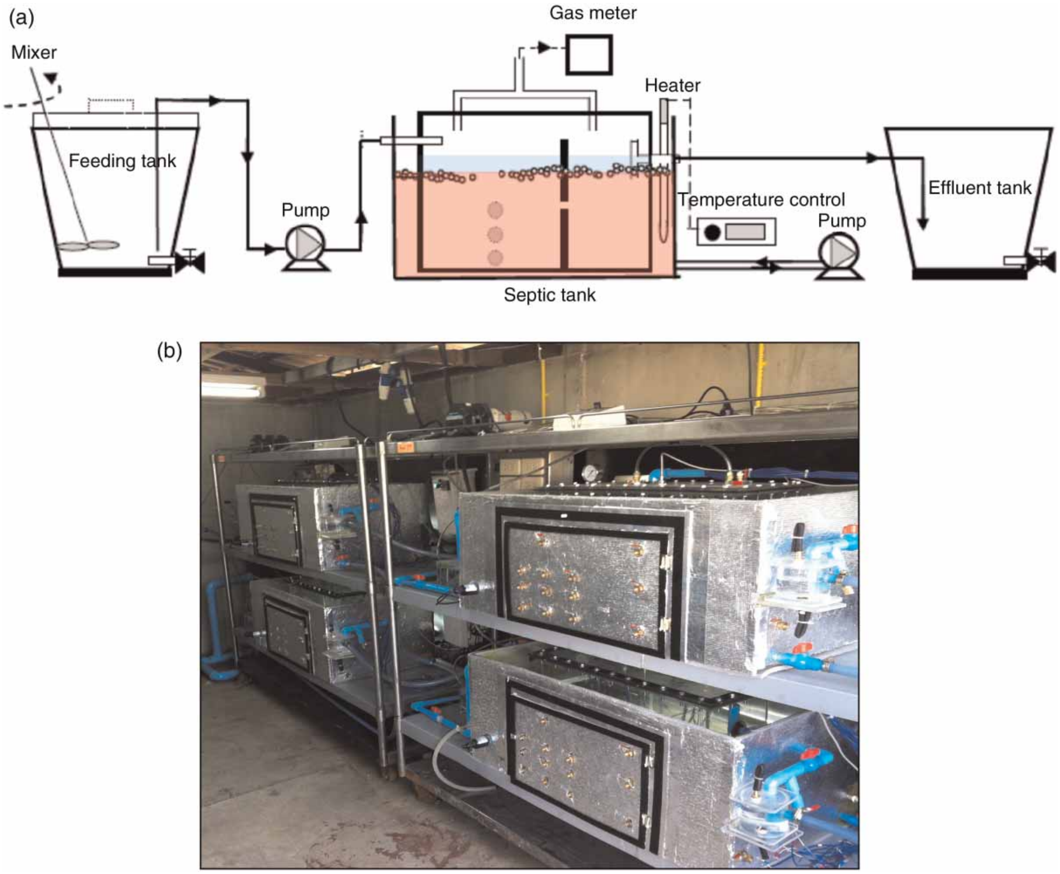

Figure 1 | Experimental set-up: (a) layout diagram; (b) laboratory-scale septic tanks at Asian Institute of Technology, Thailand.

9, 12, 24, 48, 72, 96, 120, 168 and 216 h of incubation, about 10 mL of the supernatant of each glass bottle were analyzed for *E. coli* concentrations and to determine the inactivation efficiencies.

Analytical methods

All physical and chemical analyses of the influent and effluent septage samples were done according to Standard Methods (2005). The biogas composition was determined by a gas chromatograph (Agilent 7890) equipped with FID detector, while the *E. coli* concentration was analyzed by the multiple-tube fermentation technique.

Data analysis

The data of the *E. coli* inactivation at various temperatures were used to determine an appropriate inactivation model using the first-order kinetic model (Equation (1)) and the Weibull model (Equation (3))

$$N_t/N_0 = e^{-k}T^{t} \quad (1)$$

where N_t is the number of microbial populations at any time, N_0 is the number of microbial populations at initial time, t is contact time and k_T is first-order rate constant at temperature T (Equation (2))

$$k_T = k_{20}\theta^{(T-20)} \quad (2)$$

where k_{20} is the first-order rate constant at the standard temperature of 20 °C, T is the reactor temperature and θ is a coefficient which is 1.06 (1.02–1.10, Metcalf & Eddy 2003).

Owing to various factors, the microbial inactivation may not always follow the first-order kinetic model and a modification such as the Weibull model has been proposed (Prabhakar *et al.* 2004).

$$\log N_t/N_0 = -b_T t^n \quad (3)$$

where b_T is a temperature-dependent coefficient and n is the Weibull coefficient.

The *E. coli* inactivation data obtained from the experimental study described in an earlier section were tested with Equations (1) and (2) to determine an appropriate inactivation model, which was later validated with the laboratory-scale septic tank data obtained as described above and with the data obtained from field investigation of actual septic tank performance at some household communities in central Thailand.

RESULTS AND DISCUSSION

The characteristics of septage samples used in this study are shown in Table 1. Although the average age of these septage samples was about 1 year, resulting in the BOD_5/COD ratio of about 0.32, there were still high concentrations of organic matter with the average BOD_5 and total COD concentrations of 3,250 and 10,620 mg/L, respectively. The average *E. coli* number was 9.6×10^6 MPN/100 mL, higher than the WHO guidelines for safe reuse (WHO 1989).

Table 1 | Septage characteristics

Parameter	Average value
pH	7.30 (±0.14)
Total COD (mg/L)	10,620 (±967)
Soluble COD (mg/L)	1,636 (±291)
Total BOD_5 (mg/L)	3,250 (±354)
BOD_5/COD	0.32
TKN (mg/L)	175 (±78)
TS (mg/L)	55,173 (±5,819)
VSS (mg/L)	10,947 (±1,777)
E. coli (MPN/100 mL)	9.6×10^6 (±3.8)

Note: standard deviation in parentheses.

Effects of temperature on septic tank performance

The performance of the laboratory-scale septic tanks at various temperatures after the 60 days of operation is shown in Table 2. From the online temperature sensors, there were temperature fluctuation within ±5 °C in the septic tanks depending on hourly ambient temperatures. The supernatant temperatures were about 2 °C higher than those in the septic tank sludge. The effluent *E. coli* concentrations were found to decrease with increasing temperatures; there were about 10 MPN/100 mL at the temperatures of 50 and 60 °C, suitable for discharge into a receiving water or for reuse (WHO 1989). It is apparent that the high temperatures caused some damage to the *E. coli* cells resulting in the high inactivation efficiency. The percentage removal of COD and BOD_5 were in the ranges of 84–94% and 76–94%, respectively, comparable with the performance of conventional septic tanks and anaerobic digesters (Metcalf & Eddy 2003). The major mechanisms responsible for COD and BOD_5 removal were sedimentation of settleable solids and anaerobic digestion of organic matter in the settled sludge and in supernatants. The TS concentrations of the settled sludge in the four laboratory-scale septic tanks were found to be in the range of 49,000–53,630 mg/L. It is apparent that these septic tank effluents need to be further treated to reduce the COD and BOD_5 concentrations to meet the effluent standards for disposal or reuse in Thailand (120 and 20 mg/L, respectively) (www.pcd.go.th).

In terms of biogas production, the temperature of 40 °C gave the highest biogas volume, methane content and mL CH_4/g BOD_5 removed. There was less biogas production at the temperatures of 50 and 60 °C, probably because the methanogenic microorganisms originally present in the septage could not survive at these high temperatures or because of the absence of thermophilic methanogenic bacteria in the septage used in this study. This hypothesis was supported by a parallel study of Syne (2013), using the polymerase chain reaction technique, which found the DNA concentration of methanogenic microorganisms operating at 40°C was higher than in those operating at 32, 50 and 60 °C. The FISH analysis also showed the high number of cells hybridized with EUB338 and Arc 915 at the temperature

Table 2 | Performance of laboratory-scale septic tanks at various temperatures

	Supernatant concentrations					Biogas		
Temp (°C)	*E. coli*[a] (MPN/100 mL)	Total COD (mg/L)	% Total COD removal	BOD_5 (mg/L)	% BOD_5 removal	Biogas volume (mL)	% CH_4 content	mL CH_4/g BOD_5 removed
32[b]	$4.6 \times 10^3(\pm 2.02)$	640(±22)	93.4(±0.21)	181(±10)	94.4(±0.32)	870	40.5(±7.4)	2.8
40	$1.6 \times 10^3(\pm 0.28)$	696(±15)	94.0(±0.15)	208(±12)	93.5(±0.38)	1,394	45.9(±14)	6.7
50	12.3(±0.36)	1,144(±73)	89.2(±0.69)	438(±17)	86.5(±0.54)	781	38.8(±9.9)	2.6
60	13.4(±0.56)	1,324(±28)	84.8(±0.27)	760(±6)	76.5(±0.18)	809	33.8 (±15.7)	2.7

Note: standard deviation in parentheses.
[a]Observed on 20th day of operation.
[b]Average ambient temperature.

of 40 °C, whereas the number of these cells was much lower at 50 and 60 °C. The ratios of mL CH_4 per g BOD_5 removed as shown in Table 2 were less than the theoretical value of 350 mL CH_4 per g BOD_5 removed (Polprasert 2007) because the septage contained a low BOD_5/COD value of 0.32 (Table 1). Since, in principle, higher temperatures should promote higher bacterial activities and consequently higher biogas production, it is recommended that septic tanks operating at temperatures of 50–60 °C should be seeded with thermophilic sludge or more acclimation time should be provided to support the growth of thermophilic methanogenic bacteria.

The above results show the increase of *E. coli* inactivation efficiencies in the laboratory-scale septic tanks with increasing temperatures from 30 to 60 °C, which were later used for validation of the Weibull model. It should be noted that this study emphasized mainly the effects of temperatures on *E. coli* inactivation in septic tanks and, to maintain the same experimental conditions, no additional seeding of thermophilic bacteria was provided for the laboratory-scale septic tanks operating at 50 and 60 °C. This could be a reason for the low COD reduction and biogas production in the laboratory-scale septic tanks operating at the high temperatures.

E. coli inactivation efficiency

The experimental data for *E. coli* inactivation in the 50 mL glass bottles at temperatures of 32, 40, 50, 60 and

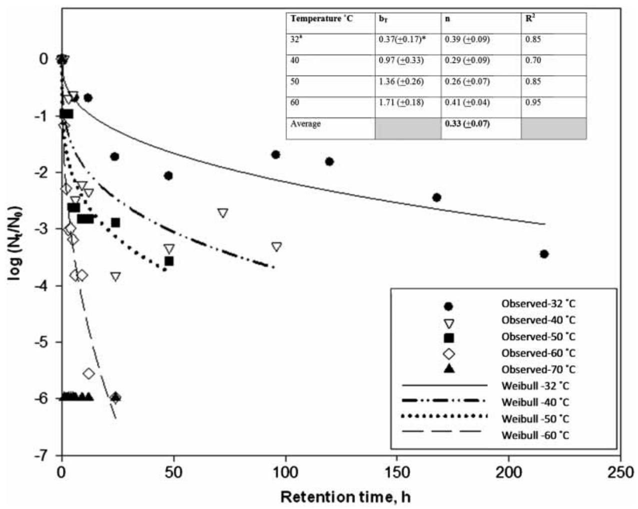

Figure 2 | Relationship between *E. coli* inactivation and retention times at various temperatures.

70 °C and retention times up to 216 h were plotted according to the Weibull model (Equation 3) as shown in Figure 2. Probably because of the complex interactions among the septage solids, *E. coli* cells and temperatures in the experimental bottles, the *E. coli* inactivation data were not found to follow the first-order kinetic model (Equation 1). Owing to the efficient heat distribution in the 50 mL glass bottles, the temperature of 70 °C resulted in immediate inactivation of the *E. coli* cells and the inactivation data at this temperature were not used in the Weibull model calculation. The values of b_T and n (applicable within the temperature range of 30–60 °C) and the correlation coefficient (R^2) of the Weibull model are given in Figure 2. From the average n value of 0.33, the

Weibull model of *E. coli* inactivation can be written as shown in Equation 4.

$$\log N_t/N_0 = \mathrm{b}_T\, t^{0.33} \quad (4)$$

A relationship between b_T and T derived from Figure 2 is shown in Equation (5) with the R^2 value of 0.97.

$$\mathrm{b}_T = 0.05\,T - 1.02 \quad (5)$$

Equation (4) was validated with the *E. coli* inactivation data obtained from the laboratory-scale septic tanks as shown in Figure 3. It is apparent that Equation (4) did not fit well with the laboratory-scale data with standard error of the estimate (SEE) of 0.84–1.63. The discrepancy was probably due to the inefficiency of heat diffusion into the *E. coli* cells and scale-up effects. To compensate for this discrepancy, a regression factor, γ, was incorporated into Equation (4) and a modified Weibull model is proposed as shown in Equation (6)

$$\log N_t/N_0 = -\gamma\, \mathrm{b}_T\, t^n \quad (6)$$

From trial and error, the γ value of 0.5 appeared to fit best the laboratory-scale *E. coli* inactivation data with SEE values of 0.40–0.82, lower than those of Equation (4) (Weibull model), as shown in Figure 3. The modified Weibull model appeared to be able to predict the *E. coli* inactivation efficiency in the laboratory-scale septic tanks operating at the temperatures of 32, 40 and 50 °C satisfactorily, but not at the temperature of 60 °C, probably owing to several factors such as efficiency of heat diffusion and response of *E. coli* cells, which may not be linear with increasing temperatures. Investigations on heat transfer and hydraulic mixing at various temperatures are being conducted and will be reported later.

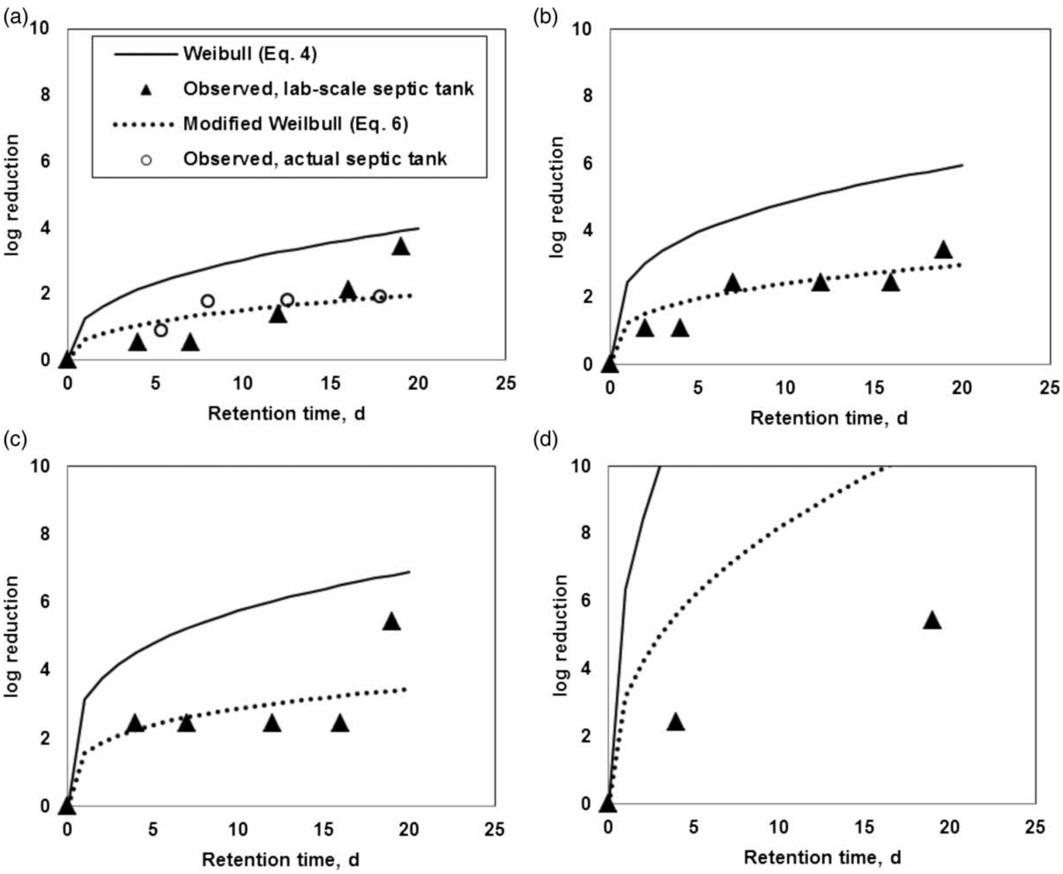

Figure 3 | Validation of modified Weibull model with data from laboratory-scale and actual septic tanks: (a) laboratory-scale and actual septic tanks, 32 °C; (b) laboratory-scale septic tank, 40 °C; (c) laboratory-scale septic tank, 50 °C; (d) laboratory-scale septic tank, 60 °C.

The modified Weibull model (Equation 6) was further validated with field data from five septic tanks belonging to some household communities in Pathumthani province, central Thailand (ambient temperature about 32 °C). It should be noted that in Thailand and in most other developing countries, only black water (excreta and flush water) is discharged into septic tanks, while grey water (wastewaters from washing, bathing and cleansing, etc.) is disposed of separately into nearby storm sewers or canals. This practice usually makes the retention time (or contact time) of the septic tank 5–20 days, longer than the conventional retention time of 1–3 days.

As shown in Figure 3, the predicted results of *E. coli* inactivation calculated from Equation (6) fit satisfactorily with the field data with a correlation coefficient (R^2) value of 0.68. It is suggested that the modified Weibull model (Equation 6) should be validated further with *E. coli* inactivation data from actual septic tanks operating at temperatures above and below 32 °C.

Although it is well known that increasing temperatures will result in better pathogen inactivation, there have been no data about the extent of pathogen inactivation in thermal septic tanks which could be used in the design and operation to protect public health.

It can be seen from the data shown in Table 2 and Figures 2 and 3 that the extent of *E. coli* inactivation in the laboratory-scale and actual septic tanks was less than those found in composting piles as reported by Feachem *et al.* (1983). For example, although the temperature of 62 °C in a composting pile would be able to inactivate most pathogens in about 1 h, according to Equation 6, it would require about 5 days to inactivate more than 6 log *E. coli* in the septic tanks. The *E. coli* cells could survive longer in the septic tanks because they are protected from sun (UV light) and there are more nutrients and moisture than in compost piles, which could also contain first level and second level consumers such as protozoa or beetles that consume the *E. coli* cells (Polprasert 2007).

Application of results

The experimental results obtained from this study suggest that the effluent of septic tanks could be made to contain relatively low concentrations of *E. coli* (pathogenic indicator) by the application of thermal treatment. In most developing countries, owing to land limitation and other socio-economic factors, septic tanks constructed in urban areas usually do not have drainage or leaching fields, but the septic tank effluent is directly discharged into nearby storm drains or the adjacent environment. Since septic tank effluents still contain high concentrations of fecal microorganisms, this practice poses significant health risks to the people who may have contact with this contaminated water or soil. From the modified Weibull model (Equation 6), by raising the temperature of a conventional septic tank to 60 °C, the *E. coli* concentration of the septic tank effluent could be reduced to less than 10^3 MPN/100 mL, at a contact time of 5 days, which is suitable for discharge or for agricultural and aqua-cultural reuse (WHO 1989). Thermal treatment of septic tanks can be achieved by solar heating or in combination with energy obtained from the biogas generated by the septic tanks themselves and future investigation on this practical aspect is recommended.

In general, excreta can contain various types of pathogenic microorganisms including viruses, protozoa and helminthic ova, especially from infected persons and/or during disease outbreaks. Because these pathogens can be more resistant to thermal inactivation than *E. coli*, further investigation of their inactivation efficiencies by thermal treatment is recommended.

It is evident from Equation (6) and Figure 3 that without thermal treatment septic tanks operating at ambient temperature of about 30 °C or below can achieve only about 2 log *E. coli* inactivation, which could result in the septic tank effluent being unsafe for disposal or reuse. Owing to the socio-economic conditions in developing countries, sewerage or drainage systems are not the solution to the sanitation problems. Therefore, efforts toward non-sewer sanitation should be encouraged to improve performance of the existing on-site sanitation systems. As reported in this study, thermal septic tanks are one of the improved on-site sanitation technologies which have high potential in pathogen inactivation and, with proper seeding and operation, could also produce biogas as an alternative energy source.

CONCLUSIONS

The results obtained from this study, which aimed to improve pathogen inactivation efficiency in septic tanks, can be summarized as follows

1. The *E. coli* inactivation efficiencies were found to increase with increasing temperatures; there was more than 6 log reduction of *E. coli* in the laboratory-scale septic tanks operating at temperatures of 50 °C and above.
2. The *E. coli* inactivation data were found to follow a modified Weibull model shown below:

$$\log N_t/N_0 = -\gamma\, \mathrm{b}_T\, t^n$$

$$\mathrm{b}_T = 0.05\,T - 1.02$$

$$\gamma = 0.5 \text{ and } n = 0.33$$

   The modified Weibull model could be applied to predict the extent of *E. coli* inactivation in actual septic tanks (temperature about 32°C) satisfactorily, but further validation of this model with *E. coli* inactivation data of septic tanks operating at temperatures above and below 30°C is recommended.
3. The percentage COD and BOD_5 removal in the laboratory-scale septic tanks operating at temperatures of 30, 40, 50 and 60 °C were found to be in the range of 84–94 and 76–94, respectively.
4. Biogas production was found to be highest at the temperature of 40 °C, while less biogas production was observed at temperatures of 50–60 °C, probably owing to low growth of the thermophilic methanogenic bacteria.

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REFERENCES

Carrington, E. G. 2001 *Evaluation of Sludge Treatments for Pathogen Reduction. Final Report*. Study contract, B4-3040, 322179, European Community, Luxembourg.

Eriksson, E., Auffarth, K., Henze, M. & Ledin, A. 2002 Characteristics of grey wastewater. *Urban Water* **4** (1), 85–104.

Feachem, R. G., Bradley, D. J., Garelick, H. & Mara, D. D. 1983 *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management*. Wiley, Chichester, UK.

Leclerc, H., Mossel, D. A., Trinel, P. A. & Gavini, F. 1977 Microbiological monitoring: a new test for fecal contamination. In: *Bacterial Indicators: Health Hazards Associated With Water, ASTM STP 635* (A. W. Hoadley & B. J. Dutka, ed.). American Society for Testing and Material, West Conshohocken, PA, pp. 21–31.

Metcalf & Eddy 2003 *Wastewater Engineering*. 3rd edn., McGraw-Hill, New York.

Mocé-Llivina, L., Muniesa, M., Pimenta-Vale, H., Lucena, F. & Jofre, J. 2003 Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. *Applied and Environmental Microbiology* **69** (3), 1452–1456.

Pandey, P. K. & Soupir, M. L. 2011 *Escherichia coli* inactivation kinetics in anaerobic digestion of dairy manure under moderate, mesophilic and thermophilic temperatures. *AMB Express* 1 (1), 1–10.

Polprasert, C. 2007 *Organic Waste Recycling: Technology and Management*. 3rd edn., IWA Publishing, London.

Prabhakar Murthy, D. N., Min, X. & Renyan, J. 2004 *Weibull Models*. John Wiley & Sons, Hoboken, NJ.

Rybczynski, W., Polprasert, C. & McGarry, M. 1982 *Low-cost Technology Options for Sanitation. A State-of-the-Art Review and Annotated Bibliography, WB ATWSS 4*. World Bank, Washington, DC.

Standard Methods for the Examination of Water and Wastewater 2005 21st edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Syne, N. E. 2013 *Determination of Microbial Dynamics and Pathogen Inactivation Efficiency from Thermophilic Septic Tanks*. Master's Thesis, Asian Institute of Technology, Pathumthani, Thailand.

WHO 1989 *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture*. Report of a WHO Scientific Group, Technical Report Series No. 778, WHO, Geneva.

WHO 2013 World Health Statistics 2013. www.who.int/gho/publications/world_health_statistics/2013/en/ (accessed 15 October 2013).

Winblad, U. & Simpson-Hébert, M. 2004 *Ecological Sanitation*. (revised and enlarged edn)., Stockholm Environment Institute, Stockholm, Retrieved from www.ecosanres.org/ES2_download.htm (accessed 15 October 2013).

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