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**DELIVERING WATER, SANITATION AND HYGIENE SERVICES
IN AN UNCERTAIN ENVIRONMENT**

**Effect of post-treatment conditions on the inactivation of
Escherichia coli after the composting process**

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A pilot study for the rural model of composting toilet with urine diversion is performed in three villages in Burkina Faso. Millet husk was used as a composting matrix. Compost made from fecal matter in this toilet has high concentration of pathogens that needs post-treatment to inactivate pathogens before using it as fertilizer or soil conditioner. Escherichia coli ATCC 11775 was used as an indicator microorganism in this study. The aim of this research is to evaluate the inactivation rate with environmental conditions such temperature, pH, and moisture content. Lab scale experiments were conducted setting environmental conditions that could inactivate Escherichia coli in post-treatment. Higher temperatures such as 50°C and 70°C with lower moisture content such as 50% can effectively inactivate Escherichia coli. Increasing the pH of the compost can also effectively inactivate Escherichia coli.

Introduction

Improving sustainable water and sanitation systems in Sahel Regions in Africa has been of concern to many organisations in the Water and Sanitation sector, because fecal matter sometimes contains pathogens that cause many water-borne diseases in the world (Singh *et al.*, 2011). There can be out breaks of diseases due to the contamination of fecal matter in water or foods caused by poor sanitary conditions. Some rural households in Africa find it difficult to manage fecal sludge sanitarly. Fecal matter is usually thrown indiscriminately in the environment and subsequently contaminates surface water and groundwater through runoff and infiltration. Isolation of fecal matter and wiping off the pathogens in the fecal matter are important processes to keep the households sanitary. Installation of latrine can achieve isolation of fecal matter from a living space. However, conventional pit latrine requires periodical withdrawing of immature fecal matter, which still contains pathogens, from the pit. The pit latrine is often left in an unsuitable condition when it is filled up, because of lack of space to dump the fecal matter.

Ecological sanitation (ECOSAN) is one of the ideas for sustainable sanitation (Langergraber and Muelleggera, 2005). They installed latrines with natural ventilation. The faeces are collected with plastic bucket and people put ash into the bucket after defecation. The fecal matter is periodically collected to the composting reactor and composted for several months (WaterAid, 2011). The composted fecal matter is then used for agricultural purpose. However, this system needs a big reactor to store compost and maintain the temperature of the compost to inactivate pathogens. This resulted in difficulty to install small scale projects.

The composting toilet system with composting matrix has been proposed for the small scale sanitation systems. In this system, the faeces are regarded as personal property since it produces compost as fertilizers. The toilet has composting reactor filled with composting matrix with capacity of water and air, window for ventilation, and rotation system. The rotation system of the reactor mixes the faeces and the matrix. The model of composting toilet for rural area in Burkina Faso has urine diverting system to avoid accumulation of moisture in the composting reactor. This system has a rapid biodegradation rate of organic matter in faeces which finishes in 2 days after final input of faeces (Lopez *et. al.*, 2004). Due to this fact, a small comparison can be made between composting reactor and the ECOSAN system. The rural model of the composting toilet is cheaper in cost than the ECOSAN system. In addition, the rural design of composting toilet has the following advantages: 1. It produces fertilizers from compost and urine, 2. It does not require

water during operation and this therefore, saves water resources, 3. Fecal matter is completely separated from the water body, 4. It requires a cheap and simple reactor, and 5. The composting process is aerobic and therefore, it produces less odour from the reactor.

A pilot study for the rural model of composting toilet with urine diverting toilet is performing in three villages in Burkina Faso namely; Kamboise, Kologondiesse and Barkoumba. The compost fertilizer produced from the composting toilet is finally reused in agriculture. We used millet husk as composting matrix, because this agricultural waste are just applied in farmland as soil conditioner. The millet husk is readily available as composting matrix for the people. We found *Escherichia coli* still remaining in the compost from preliminary experiments. This fact motivated us to minimize the health risk.

Previous studies (Koné *et al.*, 2007; Sanguinetti *et al.*, 2009; Kazama and Otaki 2011; Germer *et al.*, 2010; Kazama *et al.*, 2011; Turner *et al.*, 2005; Nakagawa *et al.*, 2005; Otaki *et al.*, 2005; Tønner-Klank *et al.*, 2007; and Niwagaba *et al.*, 2009) reported that high thermophilic temperature above 55°C can effectively inactivate pathogens. Vinnerås, *et al.* (2003) also indicated that, inactivation of pathogens can be done at high temperature. However, they also reported that, the use of ash in raising the pH can disinfect faeces. Sanguinetti *et al.*, (2009) indicated that, apart from low moisture content contributing to successful inactivation of pathogens, high pH (addition of lime) > 12 help in the inactivation process. Singh *et al.*, 2011 added that, factors such as moisture content, carbon/nitrogen ratio (C/N), particle size, pH and population of indigenous micro flora could affect the inactivation process. However, in the actual environment of the composting process, high temperatures and high pH values are difficult to achieve because they requires heat source and chemicals. Setting proper conditions for inactivation of pathogens are also proper conditions for essential organisms in the composting process. Therefore, the compost from our rural model requires post-treatment to inactivate the pathogens after the composting process in the reactor. For the post-treatment, we have the options to increase temperature by solar energy i.e. to dry compost or to add ash as alkaline.

This research specifically looked at *Escherichia coli* because it is a common indicator of pollution by faeces. The objective of the research is to determine the inactivation rate of *Escherichia coli* in possible additional treatment options after composting process such as alkaline addition (high pH), drying up with air (low moisture content) and applying solar energy (high temperature).

Methods

Compost preparation

Compost used in the experiments was collected from Kamboise pilot site in Burkina Faso. 9 people use the toilet and the compost has been used for 8 months in the site. Compost was refrigerated until use. 100g of compost was put into a sterilized bottle. Then ultra pure water was added to adjust moisture content of the compost. The combinations of temperature and moisture content for the experiments are summarized in Table 1. Temperatures were set to 37°C which is easily achieve in the actual composting toilet, and 50°C and 70°C were used as higher temperature (s) for the thermal inactivation process. Moisture contents were set to 50% (normal condition), 60% and 70% (anaerobic conditions). Here, the moisture content is defined by the following equation;

$$\text{moisture content} = \frac{\text{weight of water in the compost sample}}{\text{weight of compost sample}}$$

The bottles with compost were prepared for thermal treatment and alkaline treatment.

Bacterial culture preparation

The compost sample contained low concentration of *Escherichia coli*. For our investigations, we needed high concentration of *Escherichia coli* so that thermal treatment of *Escherichia coli* can be done for a longer period. *Escherichia coli* ATCC 11775 strains were purchased from American Type Culture Collection (ATCC) and was grown in a 10ml Nutritif Nutrient broth and incubated at 37°C over night. It was used in

No. of sample	Temperature	Moisture content
1	37°C	50%
2		60%
3		70%
4	50°C	50%
5		60%
6		70%
7	70°C	50%
8		60%
9		70%

this experiment as a model of pathogenic bacteria. The broth was kept in a refrigerator after incubation and the concentration of *Escherichia coli* was estimated.

Compost inoculation with *Escherichia coli* for thermal treatment

We inoculated compost in the bottles with 0.3ml of the broth which contains about 10^6 CFU/g of *Escherichia coli* strain and it increased the initial concentration of *Escherichia coli*. The compost was agitated for 1 minute to ensure that *Escherichia coli* were uniformly mixed. The bottles were tightly closed and immediately put into the incubators to keep temperature constant. 10g of compost sample was taken from each bottle every 2 hours. Two to three trails were done to ensure repeatability.

Bacteria extraction and measurement

Escherichia coli were extracted from compost samples using peptone water. 10g of compost sample was added to a 90ml volume of peptone water and agitated for 3 minutes. After adequate dilution (10^{-10} - 10^{-7} times) with sterilized Ringer solution, each diluted extract was inoculated in Chromocult coliform ES agar by simple layer method. The media was incubated at 37°C for one day, and then, *Escherichia coli* colonies were counted.

Alkaline treatment

For the alkaline treatment, known amount of $\text{Ca}(\text{OH})_2$ i.e. 1.0 g, 0.5 g and 0.1 g were added to 25ml of distilled water each and mixed properly. This mixture was added to the 100 g of compost with 60% of moisture content. After inoculation of *Escherichia coli*, the compost was agitated for 1 minute to ensure *Escherichia coli* were uniformly mixed. The bottles were tightly closed and immediately put at 37°C in an incubator. 10g of compost sample was taken from each bottle every 10 minutes for measurement of the concentration of *Escherichia coli*. 5g of compost sample was added into 50ml of distilled water, and then the pH of mixture liquid was measured by pH sensor.

Statistical analysis

The plate count data were converted to natural log values and subjected to analysis of variance. Data fitting for thermal and alkaline treatment of *Escherichia coli* in compost were done and the inactivation rate coefficient, k , was determined.

Results and discussions

Change in concentration of *Escherichia coli* with different temperatures

Figure 1 shows the change in the concentration of *Escherichia coli* in the compost at 37°C with 50%, 60% and 70% of moisture contents. The concentration decreased in the first 2 hours then recovered to initial level in 8 hours. All moisture content conditions followed the same pattern. Kazama and Otaki, 2011 reported that 37°C is similar to the actual temperature in the reactor of the Japanese type composting toilet. In the Japanese type toilet the inactivation rate at 37°C was very low comparing with higher temperatures. We did not find the reason for recovery of the concentration. However, treatment at 37°C is not effective for inactivation of bacteria. Figure 2 shows the time course of concentration of *Escherichia coli* at 50°C. These conditions considerably reduced *Escherichia coli* concentrations. The performance at 50°C with the lower moisture content 50% was comparatively superior. In Figure 3, there was complete inactivation within 20 minutes for 70°C 50% followed by the other conditions within 30 minutes.

Influence of moisture content and temperature on inactivation rate

Otaki *et al.* (2007) indicated that, inactivation of microorganism follows a first order reaction and it is expressed as:

$$\ln(C/C_0) = -kt$$

Where:

C : concentration of microorganism in compost sample in dry basis at time t (CFU/g-dry solid);

C_0 : initial concentration of microorganisms in compost sample in dry basis (CFU/g-dry solid);

k : inactivation rate coefficient (h^{-1});

t : reaction time (h)

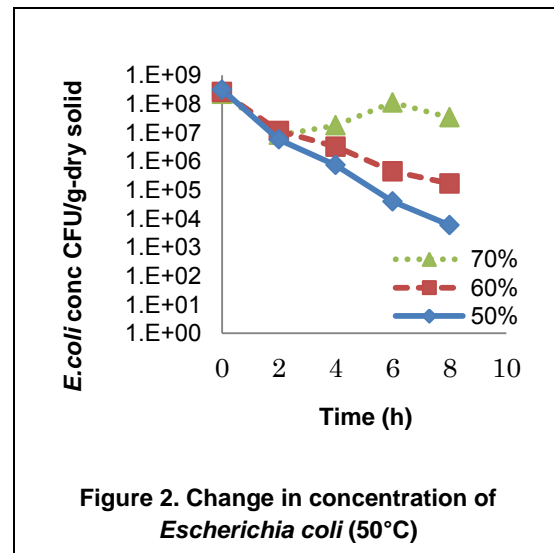
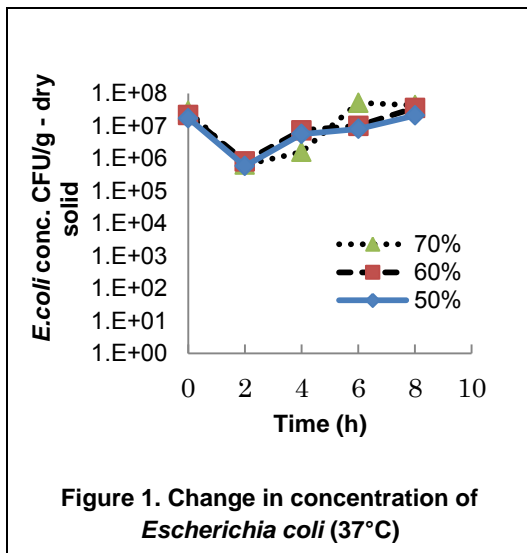
The inactivation rate coefficients were estimated by fitting the equation on the data with the least square method. Figure 4 and 5 shows the time courses of the normalized concentration of *Escherichia coli* on semi-log plot to determine inactivation rate coefficient. The time scale for 70°C were in minutes but converted to hours. The plots at 30 and 40 minutes were omitted in calculating the inactivation rate coefficients because their positions were apparently apart from the respective linear lines. The lines in Figures 4 and 5 show fitted curve of the equation. Figure 6 shows the effect of moisture content on the inactivation rate coefficient of *Escherichia coli*. Temperature and moisture content have great influence on the treatment process. Inactivation rate coefficients are more rapid in all experimental conditions with moisture content at 50%, followed by 60% and finally 70%, however there was no great difference in inactivation rate coefficient values for 50% and 60%. The results are in agreement with several studies (Kazama and Otaki, 2011; Nakagawa, N *et al*, 2005) that describe that high temperature and lower moisture content are capable of rapidly inactivating *Escherichia coli*, however we used different matrix from the studies. In our study, all treatment conditions with lower moisture content such as 50% rapidly inactivated *Escherichia coli*.

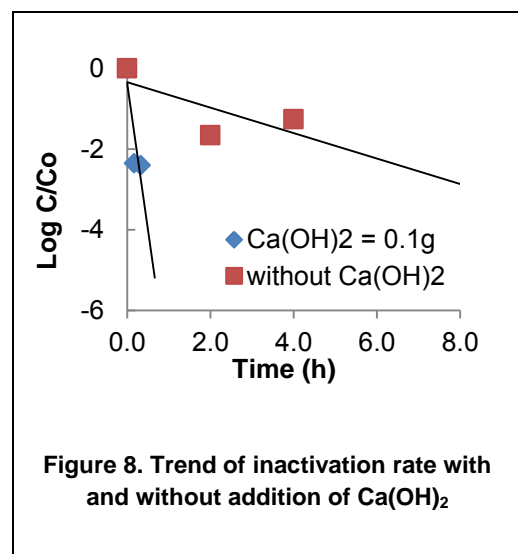
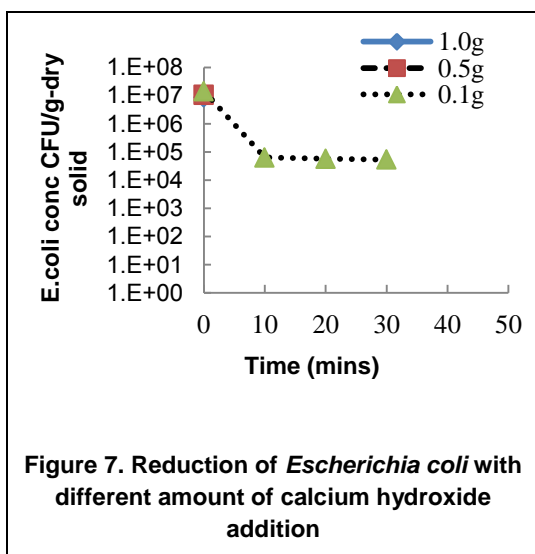
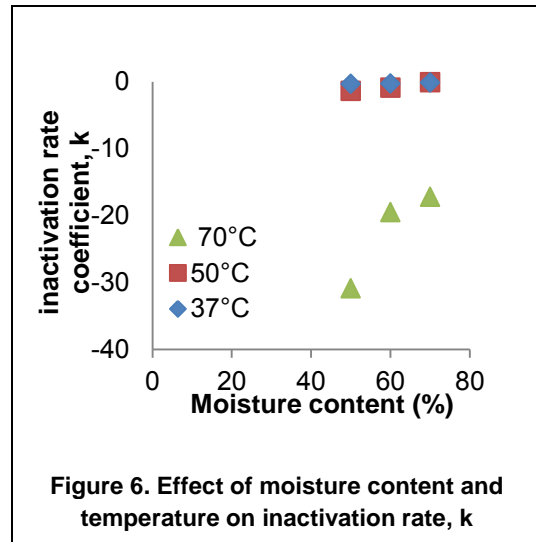
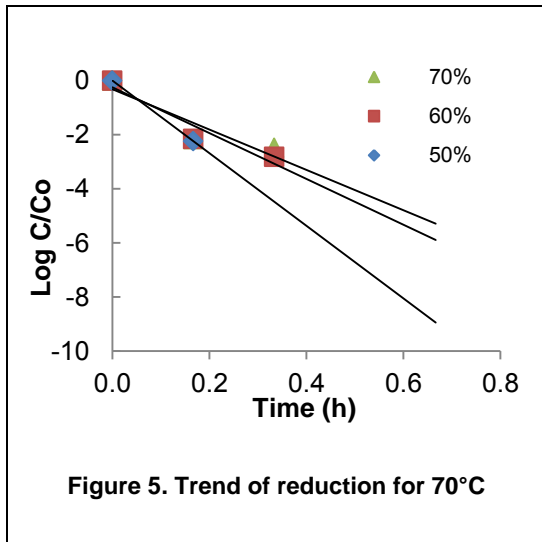
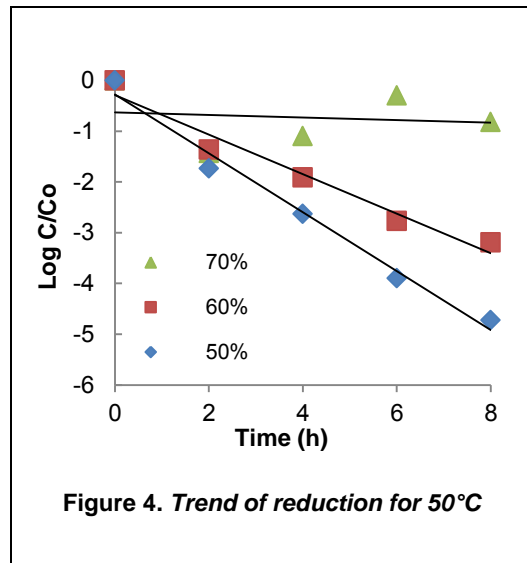
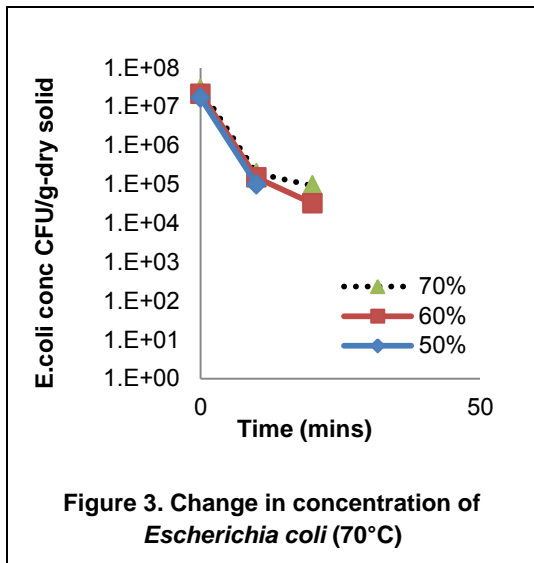
Influence of calcium hydroxide on inactivation rate

Addition 1.0 g, 0.5 g and 0.1g of Ca(OH)₂ raised the pH of compost to 8.1, 7.4 and 7.2 respectively. The inactivation rate of alkaline treatment at 37°C with 60% of moisture content is shown in Figure 7. 1.0 g and 0.5g of Ca(OH)₂ addition rapidly inactivated all *Escherichia coli* within 10 minutes, however *Escherichia coli* showed tolerance for 0.1g Ca(OH)₂ addition. Figure 8 shows the trend for inactivation of *Escherichia coli* with 0.1g of Ca(OH)₂ addition at 37°C and without addition of Ca(OH)₂. Addition of 0.1g of Ca(OH)₂ was more rapid than the without the addition of Ca(OH)₂.

Post-treatment conditions for the inactivation of *Escherichia coli*

Post-treatment of compost will allow rapid inactivation of *Escherichia coli*. Compost can be applied immediately to the soil as fertilizer. In our study, using post-treatment conditions for compost can make it safe in less than 8hrs for 50°C, 50% and 30 minutes for 70°C, 50%. For alkaline treatment, adding 0.5 g or more of Ca(OH)₂ per 100 g of compost can rapidly inactivate *Escherichia coli* within 10-20 minutes.





Conclusion

Determination of the inactivation rate of *Escherichia coli* in possible additional treatment after composting process such as alkaline addition (high pH), drying up with air (low moisture content) and heating by solar energy (high temperature) was tried with laboratory test. As a result, high temperature was capable of inactivating *Escherichia coli*. Lower moisture content during the process performed better in reducing concentration of *Escherichia coli* rather than higher moisture content. Addition of 0.5g of Ca(OH)₂ raised the pH of the compost to 7.4 and this increased the rate of inactivation. Therefore, the inactivation rate of *Escherichia coli* was increased at higher temperature such as 50°C and 70°C with lower moisture contents such as 50%. The addition of Ca(OH)₂ to the compost makes the inactivation of *Escherichia coli* more rapid.

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