Inactivation mechanism of pathogenic bacteria using lime and ash in composting toilet

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Abstract

A composting toilet, using charcoal and rice husk as matrixes, requires inactivation treatment against pathogenic microorganisms derived from human feces before agricultural reuse. High pH conditions by adding calcium oxide (CaO) and wood ash is effective for lethal inactivation of pathogenic bacteria. However, quantitative information about application is limited. Therefore this study investigated the relationship between applied amount of these and compost pH. And then, inactivation rate and damage parts of *Escherichia coli* in several alkaline level of compost were observed with three different media. Moreover the infection risk by using CaO was assessed on the assumption that four member family infected *salmonella* used a composting toilet. CaO application can increased the compost pH and it promoted inactivation of pathogenic bacteria to induce damages of outer membrane and enzyme activities in both matrixes. This application can cause the damages of nucleic acid and/or metabolism with the pH increase. Wood ash can cause damage to enzyme activity significantly but much more applied amount wsas required than CaO. By dosing 900 g of CaO and reacting four hours, it will enable to decrease the annual acceptable infection risk to 10^{-4} in both matrixes.

Keywords: composting toilet, high pH condition, inactivation mechanism, pathogenic bacteria

Introduction

Resource recycling based and small foot printed sanitation system, which is common concept of both Ecological sanitation (EcoSan) (Esrey, 1998) and Onsite Wastewater Differentiable Treatment System (OWDTS) (Lopez et al., 2002), has been expected as one solution to improve sanitation condition in developing countries. In this system, wastewater from household is fractioned into feces, urine and greywater. The fraction gives us urine as fertilizer liquid which is low hygienic risk (Pradhan et al, 2010) and irrigation water treated by simple facilities (Ushijima et al, 2012). However, reuse of feces

is required special care because of highly health risk.

A composting toilet, which is a key technology of OWDTS, has advantages for inexpensive to introduce (Ushijima *et al*., 2011) and effective to agricultural production (Hijikata *et al*, 2011a). Taking these advantages, practical installations have been attempted in urban slum in south-eastern Asia (Ushijima *et al*., 2007) and rural in sub-Sahara (Ushijima K., *et al*., 2012). In the composting toilet system, sawdust has been frequently used as composting matrix. The matrix plays a role of giving gas phase for aerial fecal decomposition with little odor. Considered to limitation of sawdust availability in all over the world, alternative matrix is necessary to diffuse the composting toilet more widely. Although chopped corn stalk, rice husk and charcoal from rice husk showed well fecal decomposition rate in the composting toilet (Hijikata *et al*, 2011b), hygienic aspect of these alternative matrixes has not been observed.

A compost from the composting toilet has a potential to trap pathogens derived from infected persons (Sossou *et al*, 2012), which raises the possibility for other users or farmers to become infected (Otaki *et al*, 2007). Therefore, inactivation treatment must be taken when the compost is exchanged to pure matrix. Although high temperature and drying with natural sunlight is effective for the bacterial reduction (Redlinger et al, 2001), the efficiency is affected by climate. As secure inactivation, alkaline treatment by adding calcium lime was practically conducted in EcoSan toilet (Günter *et al.*, 2005). Previous report by Kazama and Otaki (2011) has shown that high pH treatment by adding calcium oxide (CaO) was effective for lethal inactivation of pathogenic bacteria and viruses in short term and it is also known that wood ash can increase pH. However, information about CaO and wood ash requirement per unit compost and target pH level for the inactivation has been still limited from the viewpoint of risk assessment. Furthermore, inactivation efficiency and inactivation pattern in alternative composting matrixes such as rice husk and charcoal has not been observed.

In the present study, therefore, by using these two matrixes, the relationship between applied CaO or wood ash amount and compost pH was investigated, and then, inactivation rate and damage part of *Esherichia coli*, regarded as a model of pathogenic bacteria, in several alkaline level of compost were observed with three different media. Moreover on the assumption that CaO is applied when the matrix is changed, CaO need was calculated from the view point of risk assessment.

Methods

Composting matrix

In the present study, pig feces was used because its characteristic was similar to human feces (Lopez *et al*., 2002) and easy to obtain as an experiment. 500 - 600g (fresh base) of the feces were continuously input into 20 L of matrix in composting machines (Hitach,Ltd. BGD-120, Kinbhoshipuro GN-120) every weekday and continued the input for 1 month and 2 months. During the composting procedure, all inputted feces were monitored and moisture content of composting matrix was measured every week with dry oven at 105°C for 24 hours. Then, weight of decomposed feces and fecal decomposition

rate at the end of composting procedure were calculated from Eq. 1 and 2, which indicate fecal decomposition property (Hijikata *et al*, 2011b).

Weight of decomposed feces [g-DW] =
(Weight of inputted matrix ＋ Weight of inputted feces) － Total Weight of a composting reactor － Weight of a composting reactor）・・・（1）

Virhe.・・・（2）

Fecal load ratio which is inputted weight of feces per initial matrix weight (dry base) was also calculated as one parameter of compost character. Calcium oxide (CaO, reagent grade, Wako chemical) and as a lime wood ash (Maruta K.100 Soumokubai) were used to increase compost pH. First, the difference between CaO and wood ash was examined by adding them to 100 mL of pure water. Then the compost pH was measured with suspension after shaking with compost and water at 1:20 (w:v) ratio for 30 min.

Bacteria

In this study, *Escherichia coli* (NBRC 3301) was used as a model of pathogenic bacteria. 4% (w/w) of Tryptic Soy Broth (Difco Laboratory) was used as a growth media for *E. coli*. It was incubated in a shaking water bath at 37°C, for 20–24 hr. The *E. coli* suspension was used as inoculums for each experiment.

Extraction and counting of *E .coli*

50 g of compost was transferred to a glass bottle and autoclaved at 121°C for 15 min, and then adjust water content to 50% by sterilized deionized water. After pre-incubation of the compost at 37°C, adequate CaO and 3 g of autoclaved pig feces were mixed in the compost. 0.5 mL of *E. coli* suspension (about 10^9 cfu/g) was inoculated in the compost mixture after about ten minutes in view of reaction heat. 0.3 g of the compost mixture was appropriately sampled (0-8 h) and *E. coli* was extracted with 20 mL of 3% (w/v) beef extraction solution (Otkaki *et al*.,2002). After adequate dilution (10-10^4 times) by phosphate buffer, 1 mL of the each extracts was inoculated in three types of agar media which were Tryptic Soy Agar (TSA), Desooxycholate Agar (DESO) and Compact Dry EC (C-EC). These media were incubated at 37°C for one day, and then, count colonies. It was reported that the recoveries of *E. coli* using this method were 70-100% (Otaki *et al*., 2002).

Calculation on inactivation rate of *E. coli*

According to previous studies (Otaki *et al*., 2007), the inactivation of microorganism followed

equations as follow. Inactivation rate constant in each media were separately calculated with colony number of each sampling time. Normalized inactivation rate constant, which was mean value of inactivation rate constant with three media, were also calculated by the results of 3 media.

$$\ln\left(\frac{N}{N_0}\right) = -kt \quad \cdot\cdot\cdot (3)$$

N : concentration of microorganism at time t;
N_0 : concentration of microorganism at time 0;
k : inactivation rate constant t : time

Estimation of damage to *E. coli*

Followed by a report of Kazama et al (2011), damage part of E. coli was estimated with inactivation rate constant on each media. The damage to *E. coli* can be shown in Table 1.

TSA is a non-selective agar and it can detect bacteria which can metabolize protein. Un-detection on TSA indicates that the nucleic acid and/or metabolism of bacteria have been damaged.

C-EC is a selective agar for E. coli and it can detect the bacteria which can secrete β-glucuronidase. Undetection on C-EC indicates that enzyme activity has been damaged.

DESO is a selective agar for E. coli and it can detect the bacteria which can metabolize lactose in the presence of desoxycholic acid. Gram-positive bacteria are unable to grow in the presence of desoxycholic acid because they lack an outer membrane and their growth is inhibited by its surface-active effect. The undetection on DESO indicates that outer membrane has been damaged.

Risk assessment

The common procedure of risk assessment is shown by NRC (National Research Council).We followed this procedure and assessed the pathogenic risk when using a composting toilet.

Hazard identification

In the use of composting toilet, there is a possibility that user become infected when they change matrix by oral infection. Therefore in this study, referring to the result of *E. coli*, *salmonella*, which is closely-related to *E. coli*, was used as a model (Asano *et al*., 1998).

Dose-response assessment

In this study, Beta-Poisson model was used. This model is based on the assumptions that the probability of infection depends on the number of ingested pathogens not the infectability of an ingested bacterium. Model parameter and the probability of infection are shown in Table 2.

$$P(D) = 1 - \left[1 + \left(\frac{D}{\beta}\right)\right]^{-\alpha} \quad \cdot \cdot \cdot (4)$$

P：Infection risk per one exposure which is a function of D, α, and β

D：Mean ingested dose, α, β：Model parameter

Exposure assessment

In this study we assumed the case that four member families used a composting toilet for three months, whose matrix amount is 40 mL. On this assumption, one person excrete about 150 g of feces per a day and all member are infected by pathogens (*salmonella*).The feces by stakeholder includes 10^6 cfu/g of *salmonella* (Suzuki *et al*., 1997). The amount of compost after three months is assumed to be 30,000 g-DW and it can be calculated that 1 g of matrix includes 2×10^4 cfu/g of *salmonella*. Therefore we overestimated that 1 g of compost includes 10^5 cfu/g of *salmonella*.

The compost is exchanged every three month (4 times in a year) and CaO is applied before the matrix is exchanged. The amount of oral intake was assumed 500 g per one time. Also, we assumed that new feces were never inputted as long as CaO reacted to compost.

The amount of exposure in each alkaline level was calculated on the basis of inactivation rate constant by TSA, which was obtained by Eq. (3). The inactivation rate on pH 10.1-10.5 was estimated by line approximation. The annual risk was calculated by following the equation as follow.

$$\text{Annual infection risk} = 1 - (1 - P)^n \quad \cdot \cdot \cdot (5)$$

P：Infection risk per one exposure

n：times of exposure per a year

Risk assessment

According to USEPA, annual acceptable risk of waterborne disease is set to 10^{-4}, one infection out of 10,000 per a year. Based on this standard and Eq (4) and (5), acceptable infection risk per one exposure was calculated to 3.2×10^{-5}. Also we tried to disinfection within four hours because during the reaction time a toilet was not available.

Results and discussion

Compost conditions

Table.3 shows the characteristic of compost used in this experiment and Fig. 1 shows the change of water content in each matrix. Fig 2 shows the mass change of compost. There were no significant differences in characteristic of compost.

Difference between CaO and wood ash in pH increase

Fig. 3A and 3B shows the pH increase by CaO and wood ash in pure water. It was found that CaO can increase pH much more rapidly than wood ash and this indicated that much more wood ash was required to increase pH.(To lead 100 mL of pure water pH to about 10, about 0.05 mg of CaO. However in the case of wood ash, about 1 mg was required.) Moreover at pH more than 10, in the case of wood ash, the change of pH became slighter.

Relationship between applied CaO/ wood ash amount and compost pH

Fig. 4A shows that the relationship between applied CaO amount and compost pH in two kinds of compost. In each matrix, compost pH increased linearly at the range of pH 9-12. Compared with two composts, the pH of charcoal compost was changed shaper than that of rice husk. (To lead the 1 g (dry weight) of compost to pH 10, in the case of charcoal, about 16 mg of CaO was required. However in the case of rice husk, about 23 mg of was required.)

Fig. 4B and 4C show the pH increase by using CaO and wood ash in the case of rice husk. Compared to CaO case, wood ash slightly increases the compost pH and this means more amount of wood ash is required than CaO to increase pH. (To lead the 1 g (dry weight) of compost to pH 10, in the case of CaO about 16 mg of CaO was required. However in the case of wood ash, about 2400 mg of was required.)

Relation between the difference of composting period and damage on *E. coli*

Fig. 5A and 5B show the changes in concentration of *E. coli* in the charcoal and rice husk compost on three media. Inactivation rate constants calculated using Eq. (3)

Fig. 6 shows the normalized inactivation rate constant in each matix when CaO or wood ash were applied.

In both matrixes, the normalized inactivation rate constant was increased. In the case of charcoal compost, the inactivation rate constant at pH 10.1, 10.4 was 8times, 12 times higher than that at pH 9.6 (without CaO) , respectably. On the other hand, in the case of rice husk, the inactivation rate constant at

pH 10.1, 10.5 was 2 times, 4 times higher than that at pH 9.1 (without CaO) , respectably. The differences showed that charcoal compost was more likely to be affected by alkalization using CaO and inactivation was promoted more.

When pH was increased by wood ash, it can more lethal damage to *E. coli* at the same pH level as CaO application. Inactivation rate constant at pH 10.0 by wood ash is almost equal to that at pH 10.5 by CaO.

According to the study by Kazama *et al* (2011) using sawdust as a matrix, the normalized inactivation rate constant was about 1.15 h^{-1} when the pH was adjusted to 10.01 by CaO dose. And also, in high temperature condition (50℃, pH 6.96) was about 0.7h^{-1}. In this study, the values on charcoal and rice husk at pH about 10 were 1.15h^{-1}, 0.61 h^{-1} respectably. Though the degree of inactivation varied depending on the matrix, this result showed the inactivation in high pH condition became more lethal than the thermal inactivation.

Fig. 7 shows the inactivation rate constant in each three media. In the case of charcoal, at pH more than 10.1, the inactivation rate constant on DESO and C-EC was higher than that of TSA relatively. This meant that CaO application caused lethal damage of outer membrane and enzyme activities in *E. coli*. In the case of rice husk compost, at pH more than 10.1 the inactivation rate constant on C-EC increased and at pH more than 10.5, the inactivation rate constant on DESO increased. In each matrix, the inactivation rate constant on TSA increased more slightly than that of DESO and C-EC with the pH increase. Therefore this indicated that the increase of pH cause more lethal damage by the increase of the damage parts of *E. coli*.

In the case of wood ash, the tendency of damage parts seemed to be the same as CaO application at pH 10.*E. coli* tended to get damage to their enzyme activity. However to raise the pH, much amount of wood ash was required. In this study the ratio of compost and wood ash was about 1:1. Therefore there may another effect except for pH, for example, solid material or electric conductivity or reaction heat. And also there is a possibility that water content was changed by adding much amount of wood ash, This point should be examined in more detail.

In short, it was found that CaO application promoted inactivation of *E. coli* to induce damages of outer membrane and enzyme activities and can contribute the damage of nucleic acid and/or metabolism as the pH was increased.

According to the previous study by Kazama *et al* (2011), the inactivation rate constant on all three media was about 1.15 h^{-1} and this indicated that estimated damage parts were nucleic acid and/or metabolism. This difference is assumed to be the chemical materials included matrix such as tar, phenolic constituent, lignin-degradation product, inorganic matter and so on. However it wasn't explained by this study and requires more detail study.

Pathogenic risk by *salmonella*

Fig 8A and Fig. 8B show the simulation of infection risk in several alkaline levels. The horizontal axis is the reaction time to CaO and the vertical axis is the infection risk per one exposure. And

horizontal black line is acceptable infection risk (3.2×10^{-5}) and vertical black lines is target time (4 hours).

In each matrix, CaO application can realize the decrease the risk within a half day (12 hours), about twice rapider as the case without CaO. In order to inactivate within 4 hours, in the case of charcoal, the pH more than 10.2 was required by dosing 27mg of CaO to 1g-DW of compost. Similarly, in the case of rice husk, the pH more than 10.3 was required by dosing 28 mg of CaO to 1g-DW of compost. Assuming a composting toilet which was used by four member families for 3 months, the weight of compost becomes around 30.000g-DW. Therefore the cases of charcoal and rice husk required 810mg and 840 mg of CaO, respectably. This amount was equal to 3 % of the weight of compost at mass composition.

In short, about 900 mg of CaO can realize the inactivation within 4 hours when four member families used a composting toilet for 3 month.

Conclusion

It was concluded that CaO and wood ash application can increase the compost pH and promoted inactivation of pathogenic bacteria to induce damages of outer membrane and enzyme activities. The damage to nucleic acid / metabolism became remarkable with the pH increase. However there were different effect between CaO and wood ash. CaO required lower dose to increase pH and cause damage to *E. coli* and at the same pH level, wood ash can cause more serious damage.Assuming the case of family of four people and 3 months usage of composting toilet and reduce the annual acceptable risk to 10^{-4}, by adding about 900 g of CaO and reacting for four hours, it will enable to sanitize the compost.

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