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A fate model of pathogenic viruses in a composting toilet based on coliphage inactivation

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Abstract

A composting toilet using sawdust as a matrix has the potential to trap pathogens that might occasionally be contained in human feces. Therefore, care should be taken when handling the sawdust. It should also be noted that pathogenic viruses tend to have stronger tolerance than pathogenic bacteria. The fates of several species of coliphages, T4, λ, Qβ and MS2, in sawdust were investigated as a viral model. The fates of coliphages were significantly different among them, and they changed in response to temperature and the water content of the sawdust. As the results, T4 coliphage had the strongest tolerance and Qβ had the weakest one in sawdust. It was estimated the days required to decrease virus to a safe level based on a risk assessment. According to the rates of Qβ and T4, 15 days and 167 days were required respectively for a safe level of infection risk based on actually operated composting toilet condition. Thus, it was significantly different depending on the species and sawdust conditions.

Key words: composting toilet; viral indicator; coliphage; risk assessment

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Introduction

The composting toilet uses sawdust as a matrix that enhances the decomposing of feces in aerobic conditions and decreases the volume by evaporation of water. This mechanism can realize the efficient organic decomposition of feces with few odors. This treatment requires no water or drainage, and used sawdust can be recycled as fertilizer. Therefore, compared with a flush toilet, the composting toilet has many advantages such as preserving water resources and allowing nutrient recycling. The composting toilet is useful especially for improvement in sanitation in developing countries because it does not require infrastructure and can be introduced cheaply (Zavala et al., 2004). However, sawdust has a potential to trap pathogens derived from infected persons, which raises the possibility for other users to become infected (Otaki et al., 2006). Therefore, care should be taken when handling sawdust especially since pathogenic viruses have stronger tolerance than do pathogenic bacteria.

Escherichia coli is considered to indicate fecal pollution and to be an indicator of the fate of fecal pathogenic microorganisms. Therefore, *E. coli* testing has been used to confirm the safety of potentially polluted systems. However, *E. coli* cannot be used to monitor viruses that have strong tolerance (Otaki et al., 2007). Therefore, certain coliphages are considered to be more appropriate indicators than coliform bacteria to monitor the fate of viruses in a water environment (Kaneko, 1996).

Coliphages are classified into a number of types by their shapes and nucleic acid composition (Jacquelyn et al., 2003). They have been used as model viruses in environment and water treatment processes to monitor the fate of fecal pathogenic viruses, because their characteristics are similar to most fecal pathogenic viruses, which tend to be RNA viruses with round shapes (Sobset et al., 1995). However, the potential still exists for some types of pathogens to have stronger tolerance than that of even the model viruses. Therefore, in the present study, we estimated the fates of a number of pathogenic viruses in a composting toilet and assessed differences among model viruses, by comparing the fates of several species of coliphages (2 species of DNA and RNA coliphages, respectively).

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1 Materials and methods

1.1 Measuring microorganisms

In this study, four coliphages, T4, λ, Qβ and MS2, and one bacterium, *E. coli* K12 were used. Microorganisms were purchased from NBRC (Biological Resource Center in Incorporated Administrative Agency National Institute of Technology and Evaluation). *E. coli* strains with NBRC numbers 13168, 3301, 13965 and 13168, were used as host bacteria for T4, λ, Qβ and MS2, respectively. Tryptic Soy Broth (Becton Dickinson, USA) was used as a growth medium for coliphages and *E. coli* strains. These were incubated in a shaking water bath at 37°C for 3 to 4 hr.

In order to measure the concentration of microorganisms in sawdust, microorganisms need to be extracted into a solution (Otaki et al., 2002). A 3% (*W*/*V*) beef extract solution was used as our extraction solution. Beef extract (MP Biomedicals, USA) was dissolved in deionized water and adjusted to pH 9.6 with NaOH, then autoclaved. Sawdust at 3% (*W*/*V*) was then added and agitated for 3 min to extract the sawdust microorganisms into the beef extract solution.

A 3% polypeptone solution was used to dilute the extraction solution to a suitable concentration for measuring microorganisms. Polypeptone (Wako, Japan) was dissolved in deionized water and autoclaved.

Coliphages T4, λ, Qβ and MS2 were measured by the double agar layer method (Otaki et al., 2006, Kamiko amd Ogaki, 1993) using Tryptic Soy Agar (Becton Dickinson, USA). *E. coli* was measured by a double agar layer method using Desoxycholate Agar (Eiken Chemical Co., Japan). The results were converted to the concentration per unit or plaque weight of sawdust.

1.2 Fate of microorganisms in sawdust

The fates of coliphages and *E. coli* in sawdust were compared under various conditions of sawdust temperature and water content. The sawdust temperature set at 30, 37, 40, and 50°C for the investigation of coliphage T4 and Qβ, at 30, 37 and 50°C for coliphage λ and MS2, and at 37°C for *E. coli*. The water content of sawdust set between 30% and 66.5%.

The water content of sawdust from an actual composting toilet was decreased by heating at 70°C for 3 hr and then adjusted to 50% with deionized water. Ten grams of sawdust was transferred to a sterilized glass bottle. A 0.1 mL sample of coliphage or *E. coli* stock solution was added and the sawdust was agitated for 1 min. Approximate 0.2 g of this sawdust was then added to extraction liquid and agitated for 3 min. The concentrations of coliphage or bacteria were measured with the above methods at different retention time.

1.3 Virus fate model in an actual composting toilet

In the case of a lab-scale experiment, the sawdust condition is uniform. However, in an actual toilet, the concentration of microorganisms is most likely non uniform because the sawdust temperature and water content at the different points are non uniform. Therefore, to investigate whether the fate of microorganisms in composting toilet resembled that of the laboratory experiment, the fate of T4 coliphage was investigated in an actual composting toilet from a household in Chichibu City (Saitama).

A 100-mL coliphage T4 culture solution, at 1.09×10^9 PFU/mL, was added to the composting toilet reactor (type: S50K, width: 1510 mm, depth: 830 mm, height: 953 mm, Seiwa Denko Co., Japan) from the inlet. The sawdust was mixed by turning the screw in forward and reverse directions for 2 min each. The sawdust was then mixed automatically every two hours. Approximate 0.5 g of this sawdust was put into an extraction solution and agitated for 3 min. The concentration of coliphage T4 was measured by the above method. The transport profile and the concentration change were investigated at sampling points of at the inlet, the center and that outlet at different retention time.

The water content of inlet, center, and outlet were 52%, 51% and 51% respectively, and temperature was 32°C at all sampling points.

1.4 Kinetics of microorganisms inactivaion in sawdust

According to previous studies (Nakagawa et al., 2006), the inactivation rates of microorganisms followed a first order reaction, and can be expressed as Eq. (1):

$$\ln(\frac{N}{N_0}) = -kt \tag{1}$$

where, N_0 (CFU or PFU/g) and N (CFU or PFU/g) are the concentrations of microorganisms in sawdust at time 0 and t respectively, k (hr^{-1}) is the inactivation rate constant, and t (hr) is the retention time.

2 Results

2.1 Inactivation of microorganisms in sawdust

Figure 1 shows the case where sawdust water content was 50% at 30 and 50°C. The inactivation rates of coliphages at high temperature were much faster than that at low temperature. And the inactivation rates among all types of coliphages were also different, even in the same experimental conditions. Every inactivation rate constant k was calculated and compared as an indicator of inactivation efficiency. Figure 2a, b shows the inactivation rate constants of coliphages at 30 and 50°C. Because inactivation rate constants varied with species or conditions, they are plotted logarithmically. As shown in Fig. 2, every coliphages decreased more rapidly at high temperature (Fig. 2b) than at low temperature (Fig. 2a), because the inactivation rate constants at 50°C were larger than at 30°C. Low water content was also effective at decreasing coliphages. T4 coliphage had the strongest tolerance of all coliphages used in this experiment, and Qβ coliphage had the weakest one. It was considered the trend which T4 coliphage had the strong tolerance especially at high water content.

Figure 2c shows the inactivation rate constants of *E. coli* and coliphages at 37 and 40°C, respectively. It was cleared

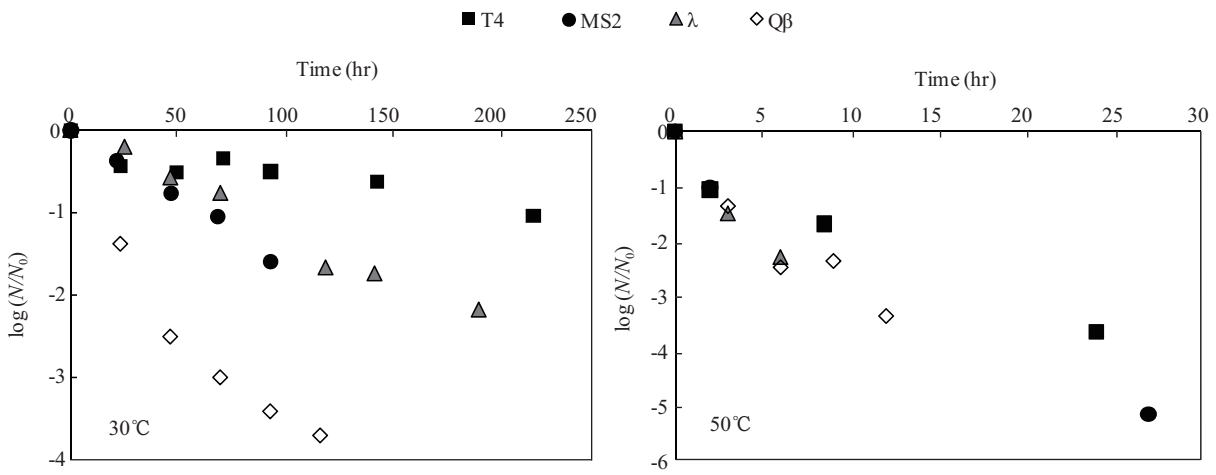

Fig. 1 Fates of coliphages in sawdust with 50% water content at 30 and 50°C.

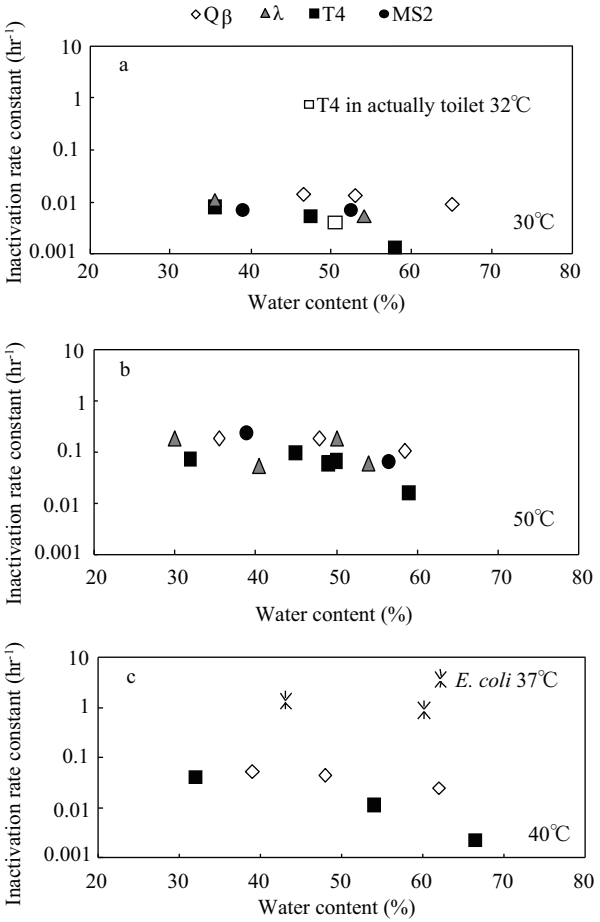

Fig. 2 Inactivation rate constants of microorganisms at different temperatures.

that *E. coli* inactivate much faster than coliphages. Therefore, it is difficult to monitor the fate of viruses in sawdust based on the fate of *E. coli*. Moreover, as the same at another temperature (Fig. 2), T4 coliphage had much strong tolerance at high water content.

2.2 Virus fate model in an actual composting toilet

Figure 3 shows the concentration change of T4 coliphage at every sampling point for the inlet, center and outlet in an actual composting toilet. For the first 48 hr, the concentrations of microorganisms fluctuated because of

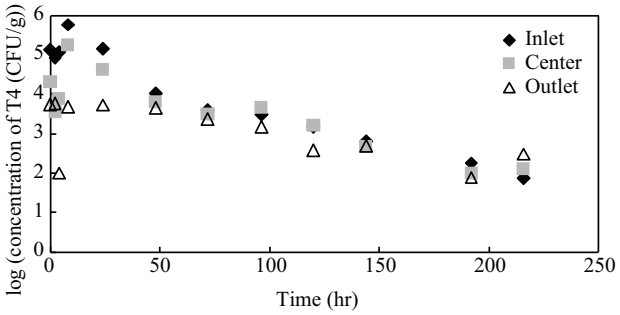

Fig. 3 Concentration change of T4 at sampling points.

non uniform mixing. In this period, concentration changes were due to both the mixing ratio and inactivation. After 48 hr, T4 coliphage was mixed uniformly. The decreases in T4 coliphage after 48 hr were due to inactivation in the sawdust. After 48 hr, the fates of T4 coliphage at all sampling points were similar. The average of the inactivation rate constants for T4 at all sampling points after 48 hr is shown in Fig. 2a. It was similar to the inactivation rate constant for T4 under a similar laboratory experiment condition (30°C, 48%). Therefore, it is considered that the fates of microorganisms in a lab-scale experiment resemble that in an actual composting toilet.

3 Discussion

3.1 Duration required for pathogen concentration in a composting toilet to decrease to a safe level

Using the inactivation rate constants, the length of time needed for the concentration of pathogens in a composting toilet to decrease to a safe level can be calculated as follows:

$$\ln(\frac{N}{N_0}) = 2.30 \times \log(\frac{N}{N_0}) = -k_e t \quad (2)$$

$$\log(\frac{N}{N_0}) = -(k_e/2.30)t \ \ (= -k_{10}t) \quad (3)$$

where, k_e and k_{10} are the inactivation rate constant expressed by natural logarithm and inactivation rate constant expressed by common logarithm, respectively.

Microbial risk assessment based on exposure assessment has been reported previously (Otaki et al., 2002; Nakagawa et al., 2006). In order to satisfy a safety level for treating compost, a removal ratio (N/N_0) is required of approximate 11.5 log, obtained by using the infection risk of a rotavirus that has a strong tolerance among enteroviruses. From this result, on which the inactivation rate constant of every coliphage was based, the number of days that corresponded to a 12 log decrease in coliphages was calculated. Figure 4 shows the days required for safe levels of infection risk at 30°C, in which microorganisms decreased the slowest in this study. The area surrounded by dotted lines in Fig. 4 shows the observed actual condition in the composting toilet operated in Chichibu City. As shown in Fig. 4, the required days estimated by the inactivation rate for each phage were significantly different. Fifteen to 167 days were required for 12 log inactivation. In the case of strongly tolerant pathogenic viruses like T4, that remain in compost especially at low temperatures (30°C) and at high water content, the standard of risk assessment should be considered carefully.

3.2 Relationship among temperature, water content, and the days required for safe levels

Figure 5 shows the relationship among temperature, water content, and the days required for a safe level of infection risk. The results showed that Qβ and T4 decreased at the fastest and slowest rates, respectively, of the coliphages used in this study. *E. coli*, as a pathogenic bacterial model, is also shown in Fig. 5 as a reference. Compared to the case of *E. coli* as a model, it always took longer for coliphages to decrease in number, even at high temperature and low water content. Moreover, the fates of coliphages were affected by both temperature and water content of the sawdust. When water content exceeded 50%, regardless of temperature, T4 in particular decreased especially slowly. Therefore, the fate of T4 appeared to be more affected by moisture than was Qβ.

Almost all enteropathogenic viruses have characteristics similar to Qβ and MS2 from the viewpoints of size, shape, nucleic acid type (RNA) and etc., so that it is possible that their fates are similar. If these enteroviruses have similar tolerance to Qβ, the region in the upper part of curve

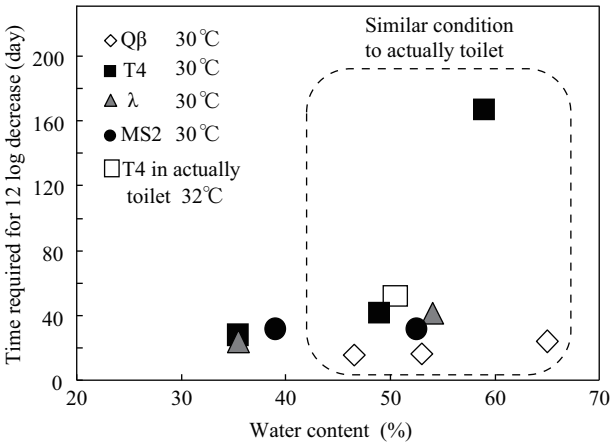

Fig. 4 Duration required for safety levels of infection risk at 30°C.

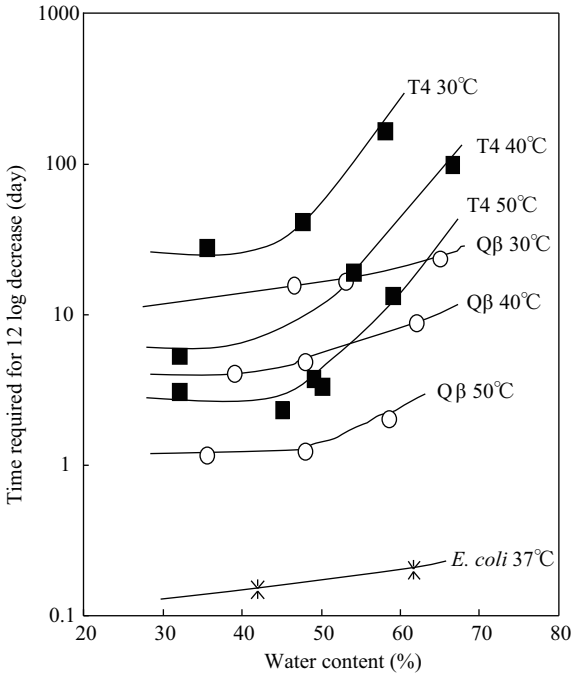

Fig. 5 Relationship among temperature, water content, and the days required for safe level.

for water content and at every temperature is considered to be a safe zone. However, for organisms like T4, the intermediate area of the value of Qβ and T4 under this condition is a gray zone. Therefore, it is questionable to use Qβ as an indicator. It is necessary to evaluate safety by considering the possible presence of pathogenic viruses with a strong tolerance like T4.

4 Conclusions

In this study, four species of DNA and RNA coliphages were used as model viruses. The infection risk posed by viruses was estimated based on the decreasing presence of coliphages. T4 and λ (DNA phages) decreased more slowly than did Qβ and MS2 (RNA phages). Their rates of decrease also depended on the sawdust temperature and water content. According to the risk assessment estimation, comparing T4 with Qβ by risk assessment, 15 to 167 days were required for 12 log inactivation, which would guarantee a safe level of infection risk. Thus, the required days for safety were significantly different depending on the species and sawdust conditions.

Most pathogenic enteroviruses have similar characteristics to Qβ or MS2, which are round shaped and have RNA genes. Their fate in compost was considered to be similar to that described here. With this assumption, although fewer days were sufficient to decrease the risk, in the case that viruses have stronger tolerance to that seen for T4, a longer or additional treatment may be necessary for safety.

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References

Zavala L, Funamizu N, Tkakuwa T, 2004. Modeling of aerobic biodegradation of feces using sawdust as a matrix. *Water Research*, 38(5): 1327–1339.

Otaki M, Nakagawa N, Akaishi F, Kubo K, 2006. The fate of microorganisms in the composting toilet from the view point of hygienic risk. In: Proceedings of the 2nd International Dry Toilet Conference. Tampere, Finland. 16–19 August. 68.

Otaki M, Nakagawa N, Kazama S, Akaishi F, Tameike S, 2007. Hygienic risk assessment and control in using composting toilet. In: The 5th International Symposium on Sustainable Sanitation. Tokyo, Japan. 19–20 September. 163–170.

Kaneko M, 1996. Suishitueiseigaku. Gihodo, Tokyo. 477.

Jacquelyn G B (Translation supervised by Hayashi H, Iwamoto A, Kamiya S, Takahashi H), 2003. Microbiology-Principles and Explorations. Maruzen, Tokyo.

Sobset D M, Battigelli A D, Handzel R T, Schwab J K, 1995. Male-specific coliphages as indicator of viral contamination of drinking water. AWWA Research Foundation and American Water Works Association.

Otaki M, Nakagawa N, Ito Y, 2002. The fate of pathogen in composting toilet and risk assessment. In: Proceedings of the 57th Annual Conference of Japan Society of Civil Engineers. Sapporo, Japan. 25–27 September. VII: 281.

Kamiko N, Ogaki S, 1993. Study for Environmental Microbial Engineering. Gihodo, Tokyo.

Nakagawa N, Oe H, Otaki M, Ishizaki K, 2006. Application of microbial risk assessment on residentially-operated biotoilet. *Journal of Water and Health*, 4: 479–486.