

Application of microbial risk assessment on a residentially-operated Bio-toilet

Naoko Nakagawa, Hana Oe, Masahiro Otaki and Katsuyoshi Ishizaki

ABSTRACT

The Sustainable Sanitation System is a new wastewater treatment system that incorporates a non-flushing toilet (Bio-toilet) that converts excreta into a reusable resource (as fertilizer or humus for organic agriculture) and reduces the pollution load to environments of the rivers, the lakes, and the sea. However, the risk of exposure to pathogens should be considered, because excrement is stored in the Bio-toilet. The aim of the present work is to analyze the health risk of dealing with the matrix (excreta and urine mixed with sawdust) of the Bio-toilet. Therefore, the fate of pathogenic viruses was investigated using coliphages as a virus index, and the modeling of the die-off rate in matrix was introduced. Then the microbial risk assessment was applied to a Bio-toilet that was actually used in a residential house; the infection risks of rotavirus and enterovirus as reference pathogens were calculated.

According to the lab-scale experiment using coliphages for investigating the die-off rate of viruses in the Bio-toilet, Q β had a higher die-off, which was greatly influenced by the water content and temperature. On the other hand, T4 showed a lower rate and was independent of water content. Therefore, these two phages' data were used as critical examples, such as viruses having high or low possibilities of remaining in the Bio-toilet during the risk assessment analysis.

As the result of the risk assessment, the storage time required for an acceptable infectious risk level has wide variations in both rotavirus and enterovirus cases depending on the phage that was used. These were 0–260 days' and 0–160 days' difference, respectively.

Key words | Bio-toilet, die-off rate, microbial risk assessment, sustainable sanitation system

INTRODUCTION

The Sustainable Sanitation System that was developed by this research is a new wastewater treatment system; it incorporates a non-flushing toilet (Bio-toilet) that converts excreta into a reusable resource (as fertilizer or humus for organic agriculture) and reduces the pollution load to environments of the rivers, the lakes, and the sea.

The Sustainable Sanitation System was launched with the support of the Japanese government named CREST, as a project called "Research on the Development of Sustainable Sanitation Systems and their Introduction in the Water Cycle System." It will be undertaken from 2002 to 2007 in Japan, with the assistance of a US\$4 million research budget

from CREST. The objective of the System is to protect human health and the environment while reducing the use of water and recycling nutrients.

The elimination of black water from the residential wastewater stream by using a non-water carriage toilet will reduce the pollution load from a household. Therefore, the Bio-toilet – which does not use water and can recycle nutrients – is one of the key components in implementing the Sustainable Sanitation System. However, the risk of exposure to pathogens should be considered, because excrement remains in the Bio-toilet for long periods of time.

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In this research, to analyze the health risk of using the Bio-toilet, the microbial risk assessment was applied to a Bio-toilet that was actually operating in a residential house; rotavirus and enterovirus were selected as reference pathogens to discuss the hygienic safety of the Bio-toilets. For the microbial risk assessment, the fate of pathogenic viruses was investigated using coliphages as a virus index; the modeling of the die-off rate was then introduced. A number of environmental conditions will speed up or slow down the time it takes for a pathogen to die, depending on the characteristics of the condition. The major factors considered important for die-off are temperature, moisture, nutrients, other organisms, sunlight and PH (Winblad *et al.* 1998). We considered die-off rate as mainly depending on temperature and moisture. Therefore, this paper also describes the modeling of the die-off rate using temperature and water content parameters concerning model viruses.

MATERIAL AND METHODS

Bio-toilet

The Bio-toilet uses sawdust as an artificial soil matrix to decompose excreta into compost, and even to form gas and humus. Inside the tank itself, a motor gently stirs sawdust or chips; in the sawdust, the water content of the excreta evaporates as a result of the heat produced by the heater and the biological activity. After the organic material is composted, it is disposed of as waste material, along with the sawdust, for use as a fertilizer. All that remains following this process are small amounts of phosphorus, minerals, salt, and humus (Nakagawa *et al.* 2001).

Figure 1 shows the structure of the Bio-toilet. The excreta are placed in the sawdust in the tank, where it ferments and decomposes as it is agitated slowly by a screw-shaped rotating mechanism. The Bio-toilet has a structure whereby sawdust is sent out, in small amounts, from the excreta inlet side to the removal opening on the side. A ventilation fan installed beside the Bio-toilet keeps the interior of the house almost completely free of the smell of the excreta. The fermentation and decomposition reduce the volume of the excreta until it can finally be used as fertilizer in dry conditions. One such Bio-toilet was installed in the house cooperating with this research and was used by three people.

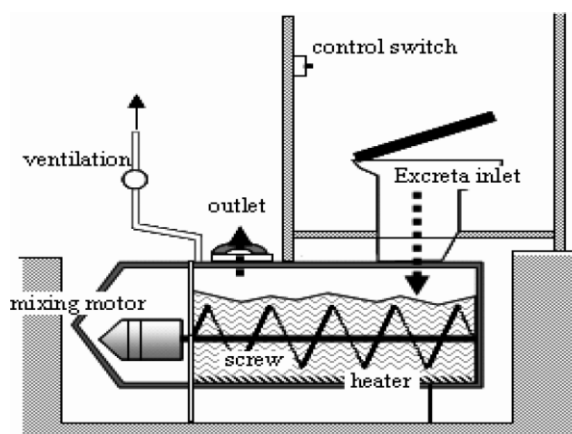


Figure 1 | The Bio-toilet.

Experimental procedure

Bacteriophage Q β and Bacteriophage T4 were selected as model viruses. After the injection of these model organisms into the Bio-toilet media, it was stirred well by hand for 5 minutes. Then amounts of between 0.1–0.2 g of the media were sampled at predetermined intervals. These samples were then injected into 10 ml 3w/v% beef extra solution (pH 9.7) and mixed well for 1 minute, in order to elute microorganisms. Coliphages were measured by a double agar method using *E.coli* K12 as a host bacteria for Q β , and *E.coli* C as a host for T4 (Otaki 2003).

The die-off rate is defined as follows.

$$-\log N/N_0 = kt \quad (1)$$

where

N : concentrations of phages [no./g],

N_0 : concentrations of phages [no./g],

k : die-off rate,

t : storage time of phage in media (in [min] or [day]).

Modeling of the die-off rate

Based on the experimental data, the effect of temperature and water content on the die-off rate (k) profiles for a range of the temperature and water content were simulated. With respect to Q β , the effect of the temperature on k was estimated by using the Arrhenius equation (Lopez *et al.* 2004) and the effect of the water content was expressed by

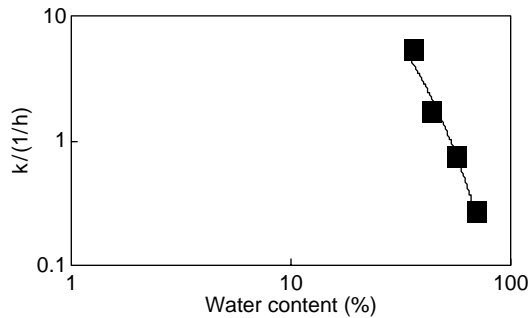


Figure 2 | The effect of water content on $K(Q\beta)$ case.

the following equation from the experimental data as shown in **Figure 2**.

$$\ln k = A \ln W + B \quad (2)$$

where

$$A = \frac{1}{\ln\left(\frac{50}{70}\right)} \ln\left(\frac{k_{50\%,T}}{k_{70\%,T}}\right)$$

$$B = \ln(k_{50\%,T}) - \ln(50)A$$

$$k_{70\%,T} = k_{s70\%} \exp\left[-\left(\frac{E_{70\%}}{R}\right)\left(\frac{1}{T+273} - \frac{1}{T_s+273}\right)\right]$$

$$k_{70\%,T} = k_{s50\%} \exp\left[-\left(\frac{E_{50\%}}{R}\right)\left(\frac{1}{T+273} - \frac{1}{T_s+273}\right)\right]$$

k_s : k at $T = 50^\circ\text{C}$ ($k_{s50\%} = 0.895(1/h)$, $k_{s70\%} = 0.274(1/h)$),

T_s : standard temperature 50 (Fujita 1995),

R : gas constant (8.314(J/mol/K)),

E : activation energy (J/mol) ($E_{50\%} = 110000$ (J/mol),

$E_{70\%} = 70000$ (J/mol)),

Then, k was expressed by temperature T and water content W as follows

$$k_{Q\beta} = k_{50\%,T} \left(\frac{W}{50}\right)^\alpha \quad (3)$$

where

$$\alpha = \frac{1}{\ln\left(\frac{50}{70}\right)} \ln\left(\frac{k_{50\%,T}}{k_{70\%,T}}\right) + \left[-\left(\frac{E_{50\%} - E_{70\%}}{R}\right)\left(\frac{1}{T+273} - \frac{1}{T_s+273}\right)\right]$$

T_4 was found to be independent of water content, from the measurement results shown in **Figure 5**. Therefore, it is

considered that only temperature effects k . Therefore, k is estimated by using just the Arrhenius equation as follows.

$$k_{T_4} = k_s \exp\left[-\frac{E}{R}\left(\frac{1}{T+273} - \frac{1}{T_s+273}\right)\right] \quad (4)$$

Being similar to the case of $k_{Q\beta}$,

k_s : k at $T = 50^\circ\text{C}$ ($k_{s50\%} = 0.06$ (1/h),

$k_{s70\%} = 0.046$ (1/h)),

T_s : standard temperature 50°C ,

R : gas constant (8.314(J/mol/K)),

E : activation energy (J/mol), ($E_{50\%} = 130000$ (J/mol),

$E_{70\%} = 80000$ (J/mol)).

Measurement of the temperature and the water content in the Bio-toilet actually operated

This Bio-toilet has been used for three years. When 30 months had passed since its installation, the temperature and the water content in the Bio-toilet were measured.

The temperature was measured two times at eight sampling points as shown in **Figure 3**.

With respect to the water content (w), the matrix was sampled seven times (once per day for one week) at each sampling point from eight locations and each was analyzed. The water content was measured by finding the weight (M) of the sawdust and the weight (M_d) after drying at a temperature of 105°C . The water content is defined by the following equation.

$$(\%) = \frac{M - M_d}{M} \times 100 \quad (5)$$

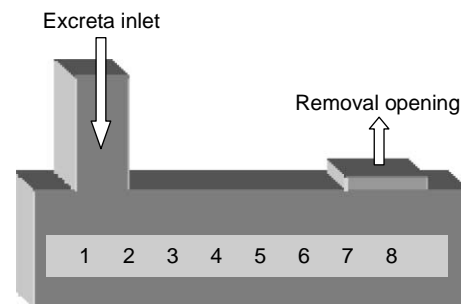


Figure 3 | The location of the sampling points.

Microbial risk assessment methods

Risk assessment is a powerful tool for evaluating the influence of hazardous agents on humans. This method consists of 4 parts. These are Hazard identification, Dose-Response assessment, Exposure assessment and Risk characterization.

Hazard Identification

From the results of the previous study, it was found that pathogenic viruses have potentially high risks and the control of them must be a key issue, rather than that of pathogenic microorganisms (Nakagawa *et al.* 2003). Therefore, rotavirus and enterovirus, which are representative waterborne pathogenic viruses, were selected as reference pathogens for this case study. According to the literature (Haas *et al.* 1999; Epstein 1998; Charles *et al.* 1999) concentrations of pathogens in feces and the duration of excretion of these pathogens are shown in Table 1. The volume of excreta is assessed to be 150 g per person, per day. The capacity of the media is 250 l. The density of the sawdust is 0.27 kg/l. The die-off rate of the pathogenic virus was based on the experimental data from the phage model described above. With respect to the period that pathogenic viruses are discharged into the Bio-toilet, it is considered to be the duration of pathogen excrement, as shown in Table 1.

Dose-response assessment

Several models that show the relation between the intake dose of the pathogen and the microbial risk have been proposed by Haas (Haas 1983), and Rose (Rose *et al.* 1991); the appropriate infective model, shown in Table 2, was used.

Table 1 | Concentration of pathogen in feces and duration of excreting pathogenic viruses (Haas *et al.* 1999; Epstein 1998; Charles *et al.* 1999).

Agent	Concentration of pathogen in feces	Duration of excreting pathogen
Rotavirus	$\sim 10^{10}$ (No./g)	5–9 days
Enterovirus	$\sim 10^6$ (No./g)	2–4 weeks

Table 2 | Parameters and models in dose-response of pathogens (Haas 1983; Rose *et al.* 1991)

Agent	Used model	Parameters
Rotavirus	β -model	$a = 0.232, \beta = 0.247$
Enterovirus	Log-normal	$GM = 250, GSD = 73$

Exposure assessment

In this house, cooperating with the research study, the residents remove matrix from the Bio-toilet and use it as a fertilizer periodically. Therefore, in this risk assessment, it was considered that resident exposure to pathogenic viruses was significant when they withdrew the compost from the Bio-toilet. Therefore, the volume of sawdust attached to the hand of the person after handling the sawdust should be estimated. In our laboratory, a survey of 14 persons was implemented. The examinee replaced the used media by hand, with and without gloves, and then the weight of the attached media was measured. We tested the media, which had three different water contents (20%, 50% and 80%).

Risk characterization

According to the results of the dose-response and exposure assessment, the infection risk of each agent was estimated using Monte Carlo techniques. The screw-shaped mixer in the Bio-toilet rotates twice to the left and twice to the right for one minute, usually every eight hours. Therefore, it is thought that pathogenic viruses in the excreta that entered the sawdust are gradually diffused while undergoing this repeated back-and-forth motion. It is important to know how long it takes the fastest-moving pathogenic microorganism to reach the removal port's opening, from the time it entered the Bio-toilet from the excreta inlet.

According to a previous study using 500 plastic balls with a diameter of 2–3 mm each, the quickest one appeared in 6 days with a median of 22 days (Kinoshita 1994). This result indicated the storage time of the pathogenic viruses in the Bio-toilet and was used in the risk assessment.

The computer simulation was performed by dividing the Bio-toilet into six sections so that the fastest-moving pathogenic microorganism was exposed for six days after

entering the Bio-toilet. In addition, a calculation was implemented where we supposed the pathogenic viruses diffused into adjacent sections equally, after being inactivated in each section.

The risk assessment was implemented in two kinds of cases. One was known as the “Actual case” where the estimated value of k in each section using the model of $Q\beta$ in the Bio-toilet was applied. On the other hand, the other case was called the “Worst case” and indicated that k in all sections of the Bio-toilet were similar to the value in the first section, where k was estimated to be at a minimum because of high water content and low temperatures (see Figure 7).

RESULTS AND DISCUSSION

Measurement results and calculated model of the die-off rate constant using the model viruses

Figures 4 and 5 show the data and model of the die-off rates of $Q\beta$ and T4, respectively.

The closed circles and squares show the measurement results. Higher temperatures caused high die-off rate in both phage cases. In the case of $Q\beta$, a dependence of water content on the die-off rate was also seen. However, it wasn't observed in the T4 case, as shown in Figure 5. $Q\beta$ is an RNA virus and T4 is a DNA virus; therefore, the difference in structure is considered to be one of the reasons for this phenomenon. However, the mechanism explaining this phenomenon hasn't yet been learned.

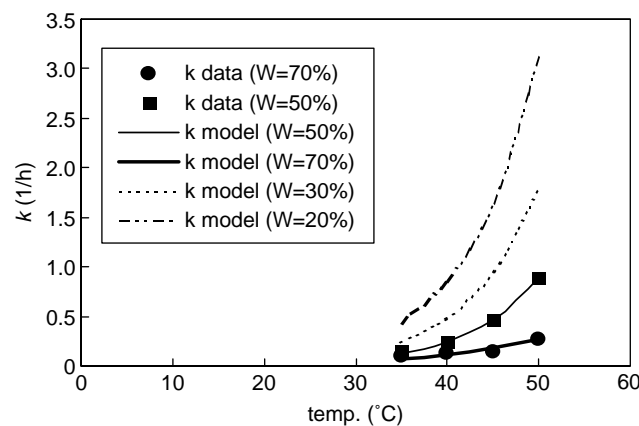


Figure 4 | The effect of temperature on die-off rate K ($Q\beta$ case).

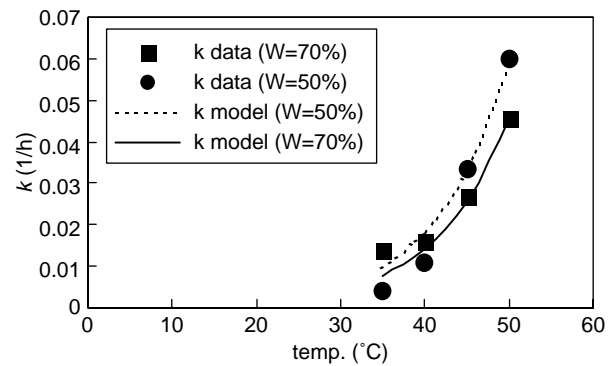


Figure 5 | The effect of temperature on die-off rate k (T4 case).

The lines in these figures show the calculated model of k concerning $Q\beta$ and T4, respectively. The line at 20% and 30% of the water content were also estimated in the case of $Q\beta$ as shown in Figure 4.

The measurement results of the temperature and the water content in the Bio-toilet actually operated

Figure 6 shows the measured temperature and water content in the Bio-toilet. The locations of each sampling point are indicated in Figure 6; they correspond to the numbers in Figure 3. The sampling point No.1 which is the point of the excreta inlet was considered to have the highest risk of a pathogen because the temperature was at the lowest, while water content was at the highest. The temperature of a middle point was around 60 degrees centigrade and it was considered that the risk of a pathogen becomes lower.

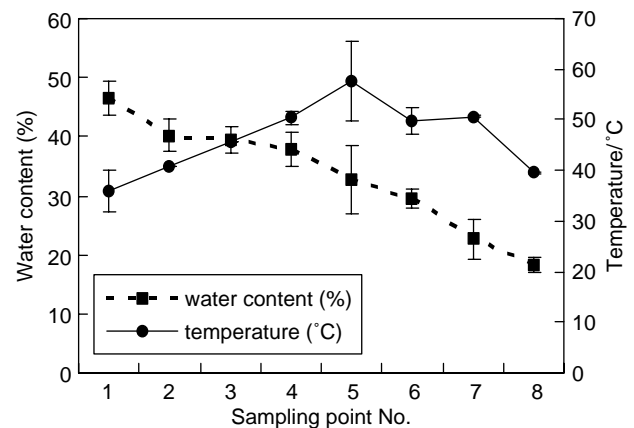


Figure 6 | Measurement result of the temperature and water content in the Bio-toilet in the house cooperating the research. (* Error bar means 95% confidential intervals).

Estimation of the die-off rate in the Bio-toilet in the house cooperating with the research

The die-off rate k of the pathogenic virus in the Bio-toilet was estimated according to the modeling of k (Equations (3) and (4)). Q β is an RNA virus and T4 is a DNA virus; both rotavirus and enterovirus are RNA viruses. In this way, the fates of rotavirus and enterovirus were assessed to be similar to that of Q β , rather than that of T4. Therefore, k of rotavirus and enterovirus in the Bio-toilet was estimated from k model of Q β , as shown in Figure 7.

Results of the exposure assessment

Figure 8 shows the results of this survey. The higher the water content was, the larger the volume of media was attached to the hand. The relationship between them was exponential, and the distribution of these volumes at each water content was assessed to have log-normal distribution, judging from a maximum likelihood method and likelihood ratio test (Haas *et al.* 1999). The median of the distribution (without gloves) was used as the volume of the exposure in the computer simulation.

Results of the risk calculation

Figure 9 shows the results of the risk calculation in the cases of rotavirus and enterovirus. Except for the worst case of rotavirus, the health risk of human infection from pathogenic viruses was almost zero less than 2.229×10^{-308} which is the minimum value which can be calculated in MS

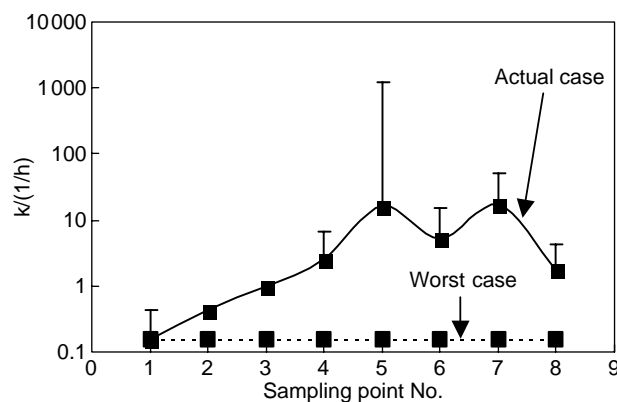


Figure 7 | Estimated k of rotavirus in the Bio-toilet. (*Error bar means 95% confidential intervals).

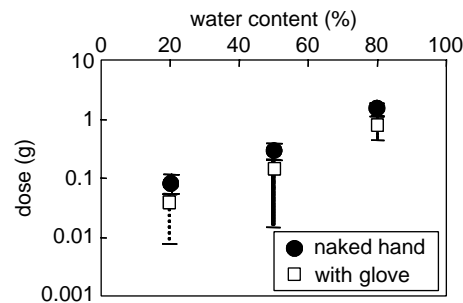


Figure 8 | Result of the survey for amount of the media attached to hand.

Excel 2003, in the case of both rotavirus and enterovirus. Even in the worst case of rotavirus, the infection risk was low. Figures 10 and 11 show the results of the risk calculation in the cases of rotavirus and enterovirus when applying the estimated value of k to each section from the model of T4. From the view point of structure, size, and type of gene, Q β was assumed to be a better indicator than T4 for rotavirus and enterovirus. However, it is necessary to consider the “worst case scenario” when using an indicator with high resistance in the Bio-toilet, like T4. Figure 10 indicates that there is a high risk when a rotavirus is discharged into the Bio-toilet; the annual risk P_{annual} due to event frequency is shown below.

$$P_{\text{annual}} = 1 - (1 - p)^n \quad (6)$$

where

p : single exposure risk,

n : frequency of event

This residence exchanges the Bio-toilet’s matrix every four months. Therefore, in order to reduce annual risk to an acceptable level (i.e. 1×10^{-4} per year (Regli *et al.* 1988), the probability of infection must be 3.33×10^{-5} .

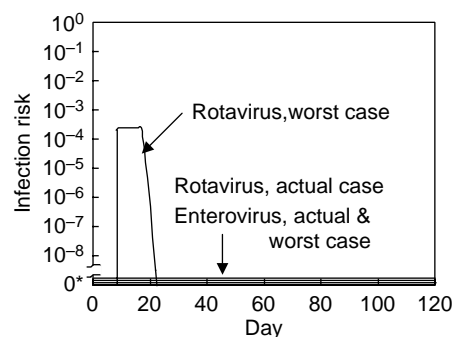


Figure 9 | Result of the risk assessment. (using Q β die-off rate, rotavirus and enterovirus case) (0* : less than minimum value which can be calculated).

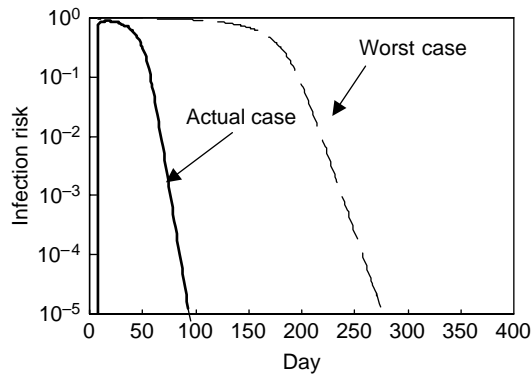


Figure 10 | Result of the risk assessment (using t_4 die-off rate, rotavirus case).

The risk decreases gradually after the rotavirus stops entering, but it takes about 70 days before the risk is set at an acceptable level, and about 260 days in a “worst case scenario”. In other words, it is best not to remove the compost for at least 90 days in this Bio-toilet, if diarrhea by rotavirus takes place to a person using this Bio-toilet and a rotavirus is discharged into the Bio-toilet. The infection risk of enterovirus decreases rapidly compared with that of rotavirus, in spite of its long period of excretion pathogenic viruses (Table 1), as shown in Figure 11. Acceptable levels of risk exposure occur after about 50 days in actual cases; however, 160 days is the conservative, “worst case scenario”. Thus, if an indicator with a low die-off rate (like T4) is used in the risk assessment, the infection risk becomes considerably high.

The storage time of the pathogenic virus required for the Bio-toilet

The die-off rate k of the model virus $Q\beta$ was used as the parameters of temperature and water content, as shown

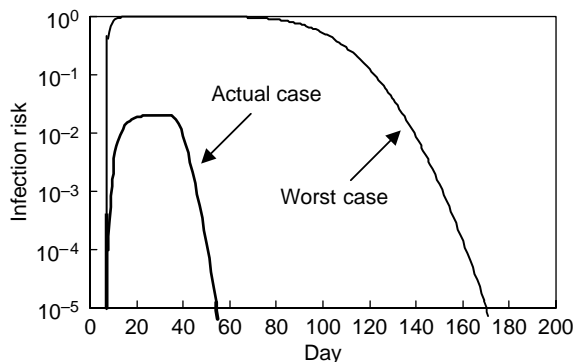


Figure 11 | Result of the risk assessment (using T4 die-off rate, enterovirus case).

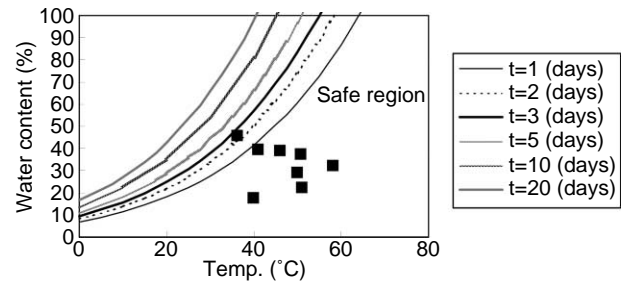


Figure 12 | The region of the safety level of the infection risk (rotavirus case using $Q\beta$ die-off rate).

in Equation (3). In the existing study, the removal ratio ($\log N/N_0$) required for the Bio-toilet is 11.5 for rotavirus and 6.5 for enterovirus, in order to hold down the annual risk to 1×10^{-4} (Nakagawa et al. 2003). There is a relation between removal ratio, the die-off rate, and storage time, as shown in Equation (1). Therefore, the required die-off rate was calculated in case of each storage time t to achieve these removal ratios by Equation (1). Then, the temperature and water content to achieve each k by Equation (3) was calculated. The result of the case of rotavirus is shown in Figure 12. The right-hand side of each curve of each storage time t indicates the region of safety levels of infection risk. The measurement results in terms of temperature and water content in each of the sample points of the Bio-toilet (as shown in Figure 3) are also shown in Figure 12 (square mark). From this figure, the required storage time is more than three days, in order to hold down the risk of infection risk for the year to 1×10^{-4} .

If the toilet is not heated mechanically, there is a risk that some of the material does not reach these high temperatures. In that case the risk will be substantially higher and a much longer storage time will be required to reach a safety level.

CONCLUSIONS

In this research, the fate of pathogenic viruses in a Bio-toilet was estimated according to the fate of coliphages. The experimental data of coliphage $Q\beta$ showed a higher die-off rate and more dependence on water content. On the other hand, that of T4 showed a lower die-off rate and was independent of water content. A risk assessment was

applied to the actual Bio-toilet condition according to the data derived in the house cooperating with the research.

As the results of the risk assessment, according to the actual data of water content and temperature in the Bio-toilet and the empirical die-off model of $Q\beta$ – which was assumed to be similar to many enteric viruses – the infection risks were estimated to be at sufficiently safe levels in this research. However, it was also indicated that there was the possibility of a high risk of infection caused by inappropriate use of the Bio-toilet in the presence of a highly resistant virus like coliphage T4.

In addition, the required storage time of the pathogenic viruses in the Bio-toilet can be estimated from the results of the risk assessment.

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