The fate of micro-organisms in the composting toilet from the view point of hygienic risk

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ABSTRACT

Composting toilet using sawdust as matrix was investigated in this research. The fate of enteromicro-organisms in the composting toilet was also investigated from the view point of hygienic risk. Several kinds of bacteriophages and enteric bacteria such as *E. coli* and *Salmonella* were injected into the sawdust matrix which had been actually used in the composting toilet. Then the concentration change of them was measured. All injected phages and bacteria were decreased in the sawdust even in 35-40 °C which should be optimal for growth of those microorganisms. In order to investigate the mechanisms of inactivation in the sawdust, various methods were applied in order to measure the damage level. The results were compared with that in the case of the other disinfection methods such as chlorination and UV. The damage level in the composting toilet was the most severe among three methods because the recovery ratio after treatment by high nutritious broth was the lowest.

Comparing the results of bacteriophages and *E. coli*s, the bacteriophages remained in the sawdust matrix for much longer time than bacteria. This result indicated that the risk of pathogenic virus was assumed to remain for long time when the infected people used the composting toilet. According to simple risk assessment, the infectious risk to other people was estimated to be below the tolerable level after several weeks.

The decreasing rate of phages and bacteria was measured in various conditions adjusted by some physical indexes such as temperature, water content and water activity. It was observed that the high temperature and low water content enhanced the decreasing rate of both of bacterio-phages and bacteria. The tendencies of dependence on water content were different in each species. Bacterio-phage Qβ had insignificant dependence on water contents. On the other hand, bacterio-phage T4 had significant dependence. Bacteria such as *E. coli* and *Salmonella* had relatively high dependence on water content. In addition, it was also observed that the water activity took an important role in determining the fate of them. Post treatment could also be suggested for reducing hygienic risk by feeding calcium oxide by changing pH.

INTRODUCTION

There are various kinds of dry toilets suggested as alternatives to water flush type [1]. The composting toilet with sawdust matrix is expected to be good alternative among them. It has various benefits such as easy structure, easy operation, cheap matrix, nutrient reusable and comfortableness. Use of porous matrix, such as sawdust, keeps aerobic conditions in toilet reactor, which suppresses bad odour and enhances the decomposition of faeces [2]. On the other hand, the composting toilet keeps faeces for certain duration. In other words, pathogenic microorganisms may be kept in composting toilet when the infected people use the composting toilet. Faeces is a high potential source of waterborne pathogenic micro-organisms, causing various symptoms such as dysentery, cholera and salmonellosis [1,3,4]. Therefore, the risk of pathogenic infection trough composting toilet should be considered.

The effect on micro-organisms in compost was reported earlier [5]. It described the required time for hygienic safety at various temperatures. It, however, didn't research the dependence of the initial concentration of micro-organisms and other physical factors such as pH, water content, etc. In composting toilet receiving the urine, water content varies and is considered as an important factor. In this study it was investigated how physical factors, such as temperature and water relating factors, influenced the fate of micro-organisms in the composting toilet. In addition, post-treatment of used sawdust matrix by calcium oxide was investigated in order to increase hygienic safety level. The idea for use of calcium oxide was referred from treatment of sludge produced in the wastewater treatment [6].

Recovery of waterborne micro-organisms exposed to conventional disinfectants such as chlorination, ozonation and UV has been reported in many papers [7,8,9]. It strongly related to inactivation mechanisms in each disinfection process and damage level of micro-organisms. Compared to these conventional disinfectants, there are few researches on the mechanisms or damage level of micro-organisms in composting toilet. In this study, the damage level of bacteria was investigated from the view point of recovery following the composting toilet treatment.

EXPERIMENTAL METHODS

Investigating the fate of microorganisms in sawdust matrix

Unused sawdust and half year old used sawdust from composting toilet were used in the experiment. The water content of these sawdust matrixes was adjusted by adding water and they were set in beakers and kept in constant temperature by incubator. Water content of the matrix was calculated with weights before and after heating at 105 °C for 3 hours in heater. Water activity was measured by water activity meter (AW SPRINT TH-500, novasina).

Four micro-organisms were selected as model enteric pathogens. *E. coli* and *Salmonella* species were used as model pathogenic bacteria. *E. coli* species (NBRC 3301) was obtained from National Institute of Technology and Evaluation (NITE), Japan. *Salmonella typhimurium* (ATCC 19585) was obtained from American Type Culture Collection (ATCC), USA. Two bacterio-phages such as Qβ and T4 were used as model pathogenic viruses. These phages categorized as coliphage group which was assumed to be a good indicator for enteropathogenic virus in environment including treatment processes due to their similar size and structure [10,11]. Bacterio-phage Qβ (ATCC 23631-B1) was F specific RNA phage and T4 (NBRC 20004) was somatic DNA phage. *E. coli* was measured by deoxycolate-agar (Eiken chemical co.ltd) and *Salmonella typh*. was measured by XLD agar (Merk co. ltd). Both phages were measured by double agar method with host bacteria. The host bacteria for Qβ and T4 were *E. coli* (NBRC 13965) and *E. coli* (NBRC 13168).

The aliquot of high density solution of the micro-organism was fed to the sawdust matrix and mixed well in beaker. In given times, approximately 0.2 g sawdust matrix was sampled and put in the 0.3 w/v% beef-extract (pH 9.7) solution for elution of microorganism. The weights of the sampled matrixes were measured precisely at every sampling time. And the concentration of micro-organism in the beef-extract solution was measured.

Investigating damage level of *E. coli* in composting toilet

E. coli (NBRC 3301) was used for investigating damage level through three kinds of disinfection treatments. Chlorination, Ultraviolet (UV) and composting toilet treatment was selected and compared each other in order to define the damage level.

Chlorination was conducted using sodium hypochlorite (NaClO) solution (Wako chem. co. ltd, Japan). The appropriate diluted NaClO solution (Wako chem. co. ltd, Japan) was prepared and the aliquot of *E. coli* solution. Sodium thiosulfate solution was added at given time for quenching chlorination. The contact time was adjusted to reach 99 % and 99.99 % inactivation rate. Tryptic soy broth (Difco co. ltd) as high nutrient was added for recovery and measurement of change in *E. coli* concentration. Ultraviolet treatment was conducted using 20 W germicidal lamp (GL-20, Toshiba co. ltd, Japan). The *E. coli* solution was exposed under UV irradiation for given time. The irradiation time was adjusted to reach 99 % and 99.99 % inactivation rate. UV irradiated solution was kept in dark to avoid photoreactivation effect [12]. The high nutrient solution was added after UV treatment and the change in *E. coli* concentration was measured. In case of composting toilet, the contact time was adjusted to reach 99 % and 99.99 % inactivation rate. Eluted *E. coli* from matrix to elution solution was put in the high nutrient solution for recovery. As reference experiment, *E. coli* solution was diluted 100 and 10,000 times which was similar concentration in 99, 99.99 %.inactivation and high nutrient solution was added.

Enhancement of disinfection effect

The unused sawdust had less disinfection effect as shown above. It means that the initial stage of operation of composting toilet has higher potential of hygienic risk. In this study, calcium oxide was used for decreasing hygienic risk even in the operation initial stage. Calcium oxide was added to unused sawdust and kept in appropriate temperature. *E. coli* was added to the sawdust and mixed well. At given time, the concentration of *E. coli* was measured. NaOH was also used for adjusting pH and *E. coli* change was investigated in same way.

RESULTS AND DISCUSSION

The fate of microorganisms in sawdust matrix

The time course change of *E. coli* and *Salmonella* in the half year old used sawdust from composting toilet is shown in **Figure 1**. These bacteria were decreased in the sawdust even though temperature (37 °C) was comfortable for the growth of both bacteria. *E. coli* was decreased as fast as *Salmonella*, and both bacteria were inactivated 99 % within a few hours. Decreasing was followed as pseudo-first order kinetics.

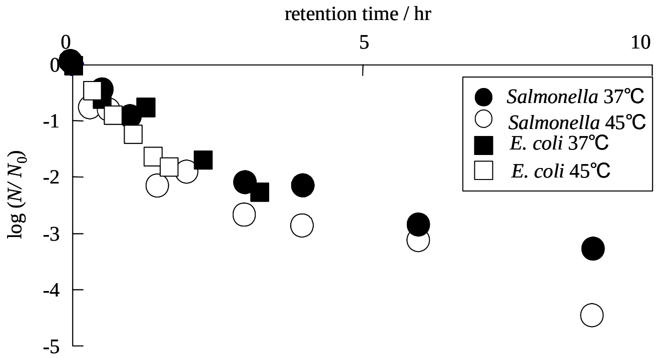

Figure 1. The time course change in *E. coli* and *Salmonella typh.* in the used sawdust From composting toilet (Temperature: 37°C, 45 °C, water content : 60%).

The time course change of bacterio phage Qβ and T4 in the half year old used sawdust from composting toilet was investigated. These phages were decreased in the sawdust, regardless of the comfortable temperature (37 °C), as well as bacteria. The inactivations in several conditions were also investigated. The inactivation of both phages could be assumed to follow the pseudo-first order kinetic. Therefore, the rate constants of these results were summarized in Figure 2. Qβ decreased faster than T4 and their rate constant was much lower than in the case of bacteria. The required time for given reduction became much longer than for bacteria cases.

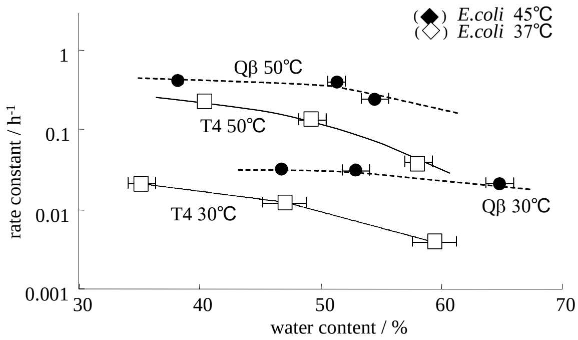

Figure 2. The time course change in Qβ and T4 phages in the sawdust used in composting toilet.

The fate of *Salmonella* in unused and used sawdust at various water contents is shown in Figures 3a and 4a. The water activity of both sawdusts in water contents can be seen in Figures 3b and 4b. It was shown that decrease of *Salmonella* in three water content conditions was different in the used sawdust matrix and that the decrease rate was faster in low water content. Water activities of the sawdust matrix in these water content cases were also different. On the other hand, there was insignificant difference among the decrease rate in three water contents' condition in unused sawdust. The water activities were also similar in three cases.

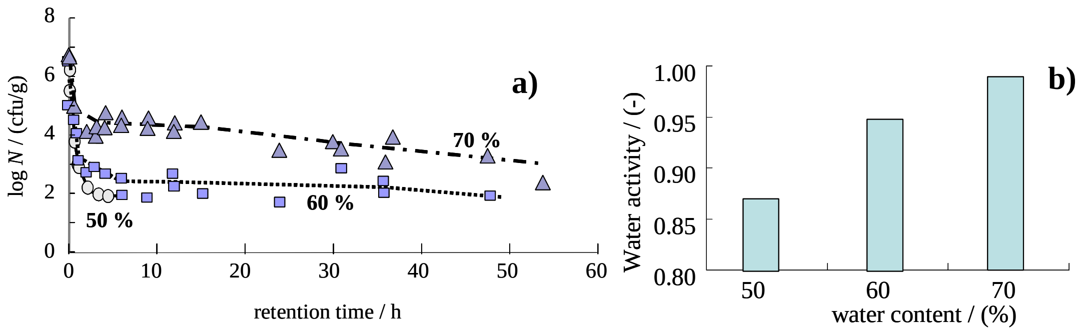

Figure 3. Change in *Salmonella* and water activities in various water conditions in used sawdust matrix.

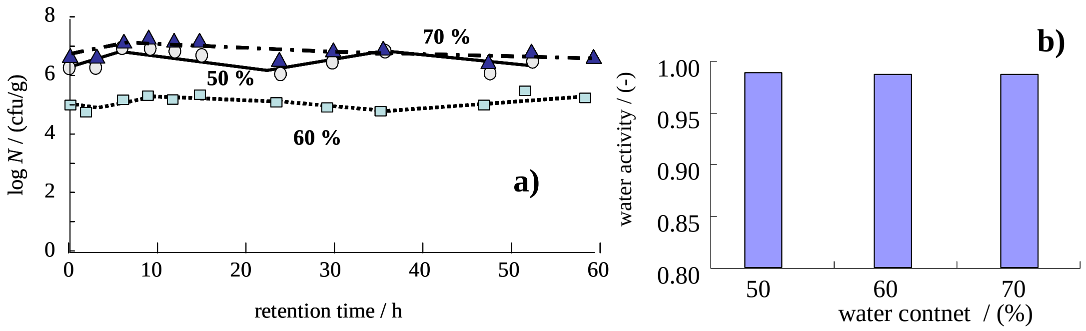

Figure 4. Change in *Salmonella* and water activities in various water conditions in no-used sawdust matrix.

Recovery of *E. coli* after composting toilet and other treatment

Change in *E. coli* in recovery solution after composting toilet treatment can be seen in Figure 5. In this figure, the result of reference experiment after dilution is also shown. *E. coli* concentration didn't increase for 4 hours in case of composting toilet even though it increased in case of reference experiment. It means that the survived *E. coli* was damaged and couldn't grow as much as undamaged (just diluted) *E. coli*. Figure 6 shows the chlorination and UV treatment cases. As shown in this figure, the recovery of *E. coli* was observed in chlorination while slight growth was observed in UV.

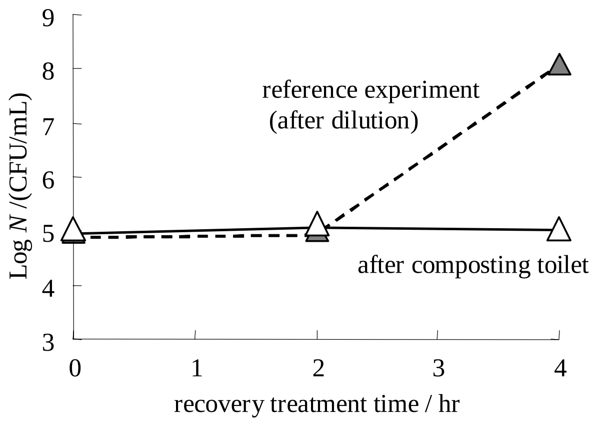

Figure 5. Recovery of *E. coli* by high nutrient solution after composting toilet treatment and change in *E. coli* in case of dilution as reference experiment.

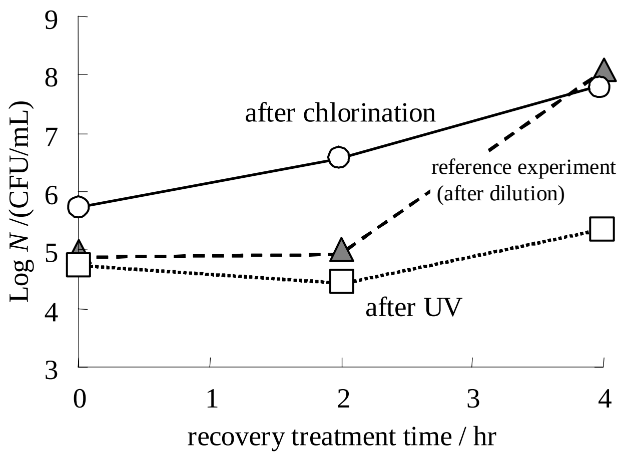

Figure 6. Recovery of *E. coli* by high nutrient solution after chlorination and UV treatment, change in *E. coli* in case of dilution as reference experiment.

Enhancement of disinfection effect by calcium oxide

The calcium oxide was added to the unused sawdust and change in concentration of *E. coli* was measured. The decrease rate was increased when much calcium oxide was added as shown in Figure 7. The addition of calcium oxide at larger amount resulted in higher decreasing rate. In this figure, pH value was also shown at various calcium oxide additions. According to pH value, it was considered that lower pH resulted in higher decreasing of *E. coli*. Figure 8 shows *E. coli* decreasing rates at various pH values, adjusted by NaOH.

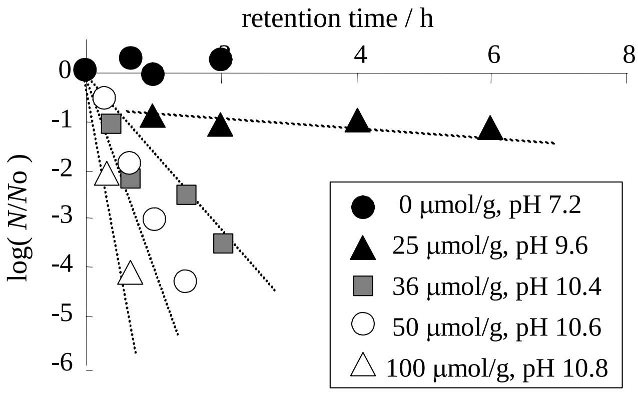

Figure 7. Decreasing rates of *E. coli* with calcium oxide at various concentration and pH.

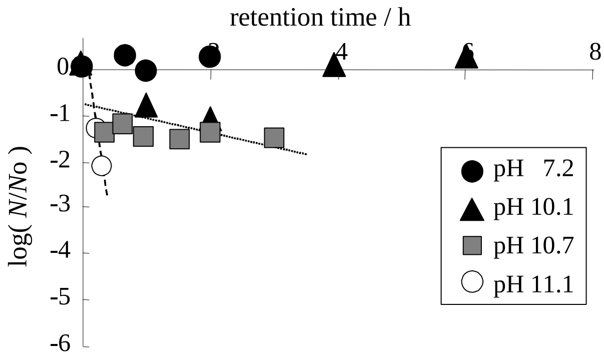

Figure 8. Decreasing rates of *E. coli* at pH adjusted by NaOH.

DISCUSSION AND CONCLUSIONS

Comparing the fate of bacteria and viruses in sawdust matrix

Coliform group including *E. coli* has been used as an indicator for estimating the efficiency in water treatment process (such as chlorination) because of higher tolerance than enteropathogenic bacteria [13]. In **Figure 1**, it was observed that inactivation rate of both bacteria was similar in composting toilet, for example 99 % inactivation occurred within a few hours. This result showed that a whole day should be enough for 99.99 % inactivation of pathogenic bacteria. And in addition, *E. coli* was considered to be potential index of pathogenic bacteria in composting toilet. Total coliform has been also used as index bacteria especially in water treatment processes. In composting toilet, the enteric bacteria contained came from only human faeces. In this case, main bacterium group detected as total coliform is assumed to be *E. coli* [13]. Therefore, it is considered that total coliform can be used as index bacteria in composting toilet.

In **Figure 2,** it was observed that bacterio-phages decreased in slower rate than *E. coli* and *Salmonella*. In some conditions, for example 37 degree C and 60 % water content, the required times for 99.99 % inactivation of Qβ and T4 were several days and a few weeks, respectively. Therefore, *E. coli* and total coliform weren't considered to be appropriate for monitoring pathogenic virus. Consequently, it was considered that *E. coli* and total coliform could be good indicators for pathogenic bacteria but not for pathogenic viruses. The alternative indicators must be considered in future studies.

Influencing factors on the decreasing rates of *Salmonella typh.* and bacterio-phages

The water content was considered to be an important factor influencing the decreasing rate of *Salmonella*. **Figure 3a** shows the dependence of decreasing rate on water content. The rate was higher in lower water content conditions. The tendency, however, wasn't observed in the case of unused sawdust, as shown in **Figure 4a**. Unused sawdust didn't contain any material originated to faeces such as organic and inorganic compounds, bacteria debris, etc. Such pure sawdust material had different features from used sawdust relating water conditions. **Figure 3 b** and **4 b** shows the water activities in both sawdust cases. Water activity means the ratio of free water and combined water. The value 1.00 of water activity means all contained water exists as free water. In **Figure 3 b**, the water activity was different in three water content cases. But in **Figure 4 b** it wasn't different. This result showed that contained water in unused sawdust remained almost as free water, and the comfortable environment to waterborne bacteria, such as *Salmonella*, remained even in low water contents. It resulted in the lower decreasing rate of *Salmonella* in unused sawdust. Consequently, the water content was an important factor on decreasing rate, but water activity should be more important in some cases such as beginning term of composting toilet operation. And in addition, the hygienic risk was considered to be higher in initial stage of composting toilet operation and the risk would be decreased during usage from the view point of practical operation.

The influencing factor in bacterio-phages cases was different from bacteria case. As shown in **Figure 2**, the water content didn't influence on the decreasing constant rate of Qβ phages. On the other hand, temperature of sawdust influenced rather than water content. Comparing with the results of bacteria cases, the inactivation mechanism was assumed to be different.

Damage level of *E. coli* in composting toilet comparing with other treatment

The difference between increased number of *E. coli* and reference experiment in **Figure 5** was smaller than in other treatment cases, as in **Figures 6** and **7**. The mechanism of chlorination was assumed to be due to chemical change of cell membrane or intercellular organs [13]. Their damage should be of wide variety. Therefore, some *E. coli* were assumed to be suffered with slight but not fatal damage. It was assumed that such slight damaged *E. coli* could be recovered in the chlorination case. The mechanism of UV treatment was elucidated as the damage on nucleic acid. The repair mechanism of damaged nucleic acid was also reported as dark repair [12]. Comparing with these treatments, the recovery of *E. coli* in case of composting toilet was significantly lower and the damage was considered to be severe and fatal due to the irreversible change of cell membrane or organs by dehydration. Consequently, it was observed that the damage of E. coli was significantly severe and fatal compared to chlorination and UV treatment.

Enhancement of disinfection effect by calcium oxide

Addition of calcium oxide was considered to be a good method for reducing hygienic risk when the used sawdust was replaced with new and used as fertilizer. The mechanism of enhancement by calcium oxide was assumed to be due to pH change. From the result of NaOH addition case, pH could enhance the decreasing rate. But its effect wasn't as much as calcium oxide case. Therefore, some additional mechanism would work in this inactivation.

CONCLUSIONS

In this study, the fate of several kinds of microorganisms in composting toilet was investigated. It was considered that *E. coli* and total coliform could be good indicators for pathogenic bacteria, but not for pathogenic viruses. The alternative indicators must be considered in future study. Water content was an important factor on decrease rate of *Salmonella*, but water activity should be more important in some cases such as initial stage of composting toilet operation. In addition, the hygienic risk was considered to be higher in such stage and. The risk must be decreased during usage from the view point of practical operation such as sawdust replacement or usage of it for fertilizer. The damage of *E. coli* in the composting toilet was also investigated. It was observed that the damage of *E. coli* was significantly severe and fatal comparing with chlorination and UV treatment.

For reducing hygienic risk, calcium oxide addition was considered to be a good method after usage of sawdust in composting toilet. The mechanism was assumed to be due to mainly pH and some unclear mechanisms.

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