

Degradation of amoxicillin in composting toilet

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ABSTRACT

We have investigated the biological and non-biological factors that affect the degradation of amoxicillin in the composting process of feces. We first examined the biological effect of living bacteria and the enzyme (beta-lactamase) on amoxicillin decay, and our results indicated that the biological effects are likely to be negligible. Consequently, we investigated the effect of phosphate and ammonia and pH level as non-biological factors by monitoring the reduction rate of amoxicillin in phosphate and ammonia buffer solutions with several pH levels. Each reduction rate constant was integrated by a simulation model, and the calculated amoxicillin reduction profile was compared to the reduction profiles of amoxicillin in the composting process of feces. The calculated results corresponded almost exactly to the experimental profiles. We therefore concluded that the degradation of amoxicillin in a toilet matrix was dependent on the concentration of ammonia, phosphate and hydroxyl ion. The phosphate and ammonia and pH level in the demonstration plant matrix were monitored, and since we found that their concentration increased, we should be able to expect the decay of amoxicillin in a toilet.

INTRODUCTION

In the Onsite Wastewater Differentiable Treatment System (OWDTS) [1], household wastewater is separated into three fractions (the blackwater, the higher load graywater, and the lower load graywater) and they can be treated separately. The blackwater is treated by a composting toilet using sawdust as a matrix. The presence of organic matter and such nutrients as N, P and K in human excrement suggests that a stabilized toilet matrix is a good candidate for effective compost. However, estrogens and a wide variety of pharmaceuticals in

human excrement are also certain to enter the reactor. Among these intruders, antibiotics in particular appear to have inhibitory effects on bacteria. One of the most frequently used antibiotics in the world is amoxicillin [2,3].

The effect of amoxicillin in human excrement on the biodegradation process of feces has been reported [4] and it was found that although the concentration of amoxicillin in toilet matrix declined quickly, it seemed to be basically difficult to treat antibiotics in a bioreactor due to their inhibitory effect on bacteria. Since the delaying effect on the feces decomposition process was strong [4], elucidation of the degradation mechanism in composting toilet seemed to be very important for enabling the stable and sound operation of the composting type toilet. This study looks further at amoxicillin degradation factors in the composting reactor.

MATERIALS AND METHODS

Evaluation of biological factors to amoxicillin reduction

The biological effect upon amoxicillin decay in the composting process can be classified into two kinds. Firstly, the bacteria in the matrix may consume the amoxicillin in the matrix (inhibition of cell wall synthesis) [5]. Secondly, amoxicillin may be enzymatically destroyed by the beta-lactamase which can be produced by wide variety of bacteria including *E. coli*. [5]. In order to evaluate these factors, we used three kinds of sawdust matrix (the ordinary toilet matrix; a matrix that contained 0.0 to 1.2 mg/g-dry clavulanic acid (beta-lactamase inhibitor.); and a gamma ray sterilized matrix). To evaluate the contribution of living bacteria in a sawdust matrix, we compared the reduction profile of amoxicillin in the ordinary matrix and that in the sterilized matrix, and, to evaluate the enzymatic decay, we compared the residual amoxicillin concentration (at 30 minutes) in a sawdust matrix that contained several levels of clavulanic acid.

The sawdust matrix used in this experiment was taken from a composting toilet that had been in operation for more than one year. Gamma ray sterilized sawdust was prepared by irradiating the sawdust with a gamma ray of 25 kGy [6]. These sawdust matrices were put into tubes with a screwcap, followed by a dissolved amoxicillin dosage and moisture content adjustment (60 %) with distilled water. They were then put into water bath (50 °C). These experimental conditions fell within the optimum temperature and moisture conditions laid down for the composting process [7,8].

Evaluation of non-biological factors for amoxicillin decay in the sawdust matrix

It has been reported that the stability of amoxicillin in an aqueous solution is affected by pH,



citric acid, boric acid, phosphoric acid, ammine and the phenolic O⁻ group, ionic strength and enzyme (beta-lactamase) [5,9,10]. Since phosphate and ammonia are supplied to the reactor in the form of human excrement, they consequently accumulate in the reactor. We supposed that phosphate, ammonia and pH in the sawdust matrix would affect the reduction of amoxicillin. To evaluate the contribution of phosphate, ammonia, and pH to the amoxicillin decay, we monitored the reduction rates of amoxicillin in the buffer solutions. The conditions of each buffer were determined on the basis of their concentrations in two kinds of sawdust matrices (SD1, SD2), described later.

Water quality analysis in the toilet matrix

Estimation of phosphate, ammonia concentration and pH

In order to estimate their concentration in the two kinds of sawdust matrices (SD1, SD2), we extracted the phosphate and ammonia by mixing the 1.4 g-wet (1g-dry) toilet matrix with 14 ml distilled water, followed by centrifugation, and we calculated the concentrations of phosphate and ammonia at the water phase in the sawdust matrix by dividing the amount extracted by 1.5 g-water, assuming that the moisture content in the sample was 60%. In this study we defined the pH of the sawdust matrix as the pH of leachate.

Estimation of EC

Since ionic strength is one of the important factors responsible for amoxicillin degradation [9], we therefore needed to estimate the ionic strength of the water in the toilet matrix; but as the estimation of the ionic strength in sawdust is difficult due to the wide variety of organic and inorganic matter in toilet matrix, we used EC as an indicator of ionic strength.

After mixing the 1.4 g-wet (1.0g-dry) of the toilet matrix with specified amount of distilled water, we measured the EC and repeated this every time more of the distilled water was added. Equation 1 was used to estimate the EC value in the sawdust matrix. The EC₀ in the equation was determined by the least-square method with experimental EC profiles. The EC of the sawdust with 60 % of moisture content was calculated as being

$$EC_{est} = EC_o \frac{V}{V'} \quad (\text{Equation 1})$$

Where

EC₀=EC of the original sawdust matrix

V= the volume of water in the sawdust matrix,

V'= the total volume of water added, in this study $VV'^{-1} = 0.266$.

Determination of amoxicillin in sawdust matrix

In order to monitor the concentration of residual amoxicillin in the sawdust, we performed extraction and clean up procedures prior to analysis [4]. The analyses were performed with a Waters Alliance system equipped with a UV detector (Waters 2487). Sample injections of 10 μ l were made with a Waters 717 plus an auto-sampler on a Atlantis[®] dC18; 3 μ m; 4.6 \times 150mm column. The separation was performed under an isocratic mode (10 mm phosphate buffer (pH4.8)/Acetonitrile=9/1) at a flow rate of 1ml with detection at 229 nm.

Monitoring a demonstration toilet

We monitored the phosphate, ammonia, and pH level in a sawdust matrix obtained from a composting toilet. The toilet (a public lavatory) is located in a river-side park in Fujishiro city, Japan. The extraction and determination methods were the same as those described in 2.3

RESULTS AND DISCUSSION

Evaluation of biological contribution to amoxicillin reduction

Consumption by viable bacteria

We monitored the changes of the amoxicillin concentration in ordinary sawdust and in sterilized sawdust to evaluate the effect of viable bacteria in a sawdust matrix (Figure 1). As Figure 1 shows, the degradation profiles of amoxicillin in ordinary sawdust and sterilized sawdust were almost the same. This indicated that the consumption to kill the viable bacteria is negligible.

Effect of the enzyme (beta-lactamase)

The beta-lactam ring in the amoxicillin structure is easily cleaved enzymatically by beta-lactamase released from various kinds of bacteria including *E. coli*. [5]. The ability of living bacteria to consume amoxicillin may be negligible, but the effect of the enzyme remaining in the matrix on amoxicillin decay cannot be ruled out. In order to evaluate the enzymatic decay, clavulanic acid was used as an inhibitor of the enzyme [5]. Figure 2 shows the effect of clavulanic acid on the degradation of amoxicillin at the end of thirty minutes. Since the amount of decomposed amoxicillin was the same as for the other samples all with differing concentrations of clavulanic acid, this result indicates that the effect of enzymatic action on amoxicillin decomposition may also be negligible. As the effect of viable bacteria and enzyme seems to be negligible, the main factors affecting the degradation of amoxicillin would appear to be non-biological.

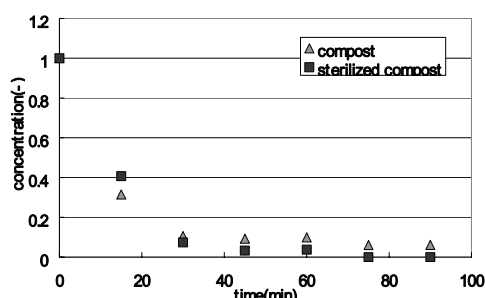


Figure 1. The change of the amoxicillin concentration in compost and sterilized compost.

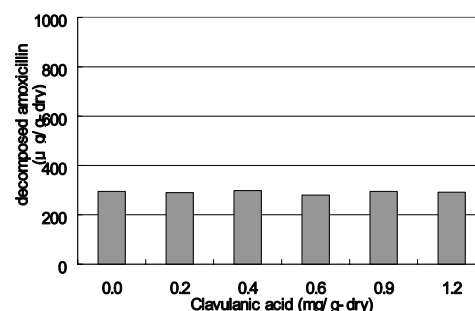


Figure 2. Effect of the clavulanic acid on the degradation of amoxicillin in compost.

Evaluation of non-biological effect on the degradation of amoxicillin in toilet matrix

Various kinds of organic and inorganic matter are supplied to a composting toilet reactor as human excrement. Among them, it was reported that phosphate, pH [11] and amines [10] accelerate the reduction of amoxicillin. To evaluate the contribution of phosphate, ammonia, and pH to the amoxicillin decay, we monitored reduction rates of amoxicillin in the buffer solutions. They were then compared with the reduction profiles of amoxicillin in the two kinds of sawdust matrix using a simulation model. We supposed that the degradation of amoxicillin would occur in the water phase of a sawdust matrix because the stability of amoxicillin in a solid state is much higher than in an aqueous solution, and the time for 10% degradation of solid amoxicillin at 20 °C was estimated being several years [9].

In order to integrate the several influencing factors, we carried out a modeling and simulation. The overall reaction was assumed to be a first order reaction for amoxicillin (Equation 2), and its rate constant includes the effect of pH, phosphate and ammonia (Equation 3). k_P and k_A are composed of the ratio of each of the ionic species and its specific rate constant (Equation 4 and Equation 5).

$$\frac{dC}{dt} = -k_{obs} C \quad (\text{Eq.2})$$

$$k_{obs} = k_{OH}[OH^-] + k_P[Total_{phosphate}] + k_A[Total_{ammonia}] \quad (\text{Equation 3})$$

$$k_P = k_{H_3PO_4} f_{H_3PO_4} + k_{H_2PO_4^-} f_{H_2PO_4^-} + k_{HPO_4^{2-}} f_{HPO_4^{2-}} + k_{PO_4^{3-}} f_{PO_4^{3-}} \quad (\text{Equation 4})$$

$$k_A = k_{NH_4^+} f_{NH_4^+} + k_{NH_3} f_{NH_3} \quad (\text{Equation 5})$$

where



k_{obs} = overall reaction rate constant
 C = the concentration of amoxicillin
 k_{OH} = the hydroxyl ion rate constant
 k_P = the phosphate rate constant
 k_A = the ammonia rate constant
 $f_{H_3PO_4}$ = molar fraction of unionized phosphoric acid
 $f_{H_2PO_4}$ = molar fraction of dihydrogen phosphate ion
 f_{HPO_4} = molar fraction of monohydrogen phosphate ion
 f_{PO_4} = molar fraction of unprotonated phosphate ion
 f_{NH_3} = molar fraction of ammonia

$f_{\text{NH}_4^+}$ = molar fraction of ammonium ion

Case 1: Constant pH, variable phosphate concentration

We prepared the phosphate buffer solutions, and we monitored the degradation rates of amoxicillin in the phosphate buffer solutions (Data not shown). The results indicate that in phosphate buffer solutions the decay of amoxicillin proceeds faster in proportion to the increasing buffer concentration. The reduction profiles of amoxicillin also indicate that with respect to the amoxicillin concentration reduction of amoxicillin seemed to follow a first-order reaction. The first-order rate constants obtained from the experiment are summarized in Figure 3a.

As for phosphate in this pH range, dihydrogen and monohydrogen phosphate ions appear to be predominant [12]. Thus the following formula should be valid.

$$k_{obs} = k_{OH^-} [OH^-] + (k_{H_2PO_4^-} f_{H_2PO_4^-} + k_{HPO_4^{2-}} f_{HPO_4^{2-}}) [Total_{Phosphate}] \text{ (Equation 6)}$$

According to Equation 6, a plot of k_{obs} against total phosphate concentration should give straight lines with the slope k_P and an intercept $k_{OH}[OH]$. Taking the dissociation constant of phosphoric acid [12] and experimental k_P value (1.20×10^{-5} , 1.22×10^{-5}), we found that the rate constants ($k_{H_2PO_4^-}$ and $k_{HPO_4^{2-}}$) were 1.15×10^{-5} and 1.22×10^{-5} $l \text{ mmol}^{-1} \text{ min}^{-1}$, respectively.

Case 2: Constant pH, variable ammonia concentration

We monitored the degradation rates of amoxicillin in the ammonia solutions. The results indicated that (Data not shown), in ammonia, the decay of amoxicillin proceeds faster in proportion to the increasing ammonia concentration. The reduction profiles of amoxicillin also indicated that with respect to the amoxicillin concentration the reduction of amoxicillin seemed to follow first-order reaction. The first-order rate constants obtained from the experiment are summarized in Figure 3b. As for ammonia, since both ammonium ion and ammonia appear to be predominant [13] in the pH region, the following formula ought therefore to be valid.

$$k_{obs} = k_{OH^-} [OH] + (k_{NH_4^+} f_{NH_4^+} + k_{NH_3} f_{NH_3}) [Total_{Ammonia}] \text{ (Equation 7)}$$

According to Equation 7, a plot of k_{obs} against total ammonia plus an ammonium ion concentration should give a straight line with a slope of k_A and an intercept of $k_{OH}[OH]$.

By using the dissociation constant [13] and the experimental k_A value (3.53×10^{-6} , 1.81×10^{-5}), we found that the rate constant k_{NH_3} and $k_{NH_4^+}$ were 3.74×10^{-5} and 4.28×10^{-7} $l \text{ mmol}^{-1} \text{ min}^{-1}$.

$\text{mmol}^{-1} \text{min}^{-1}$ respectively.

Effect of pH on amoxicillin degradation

The intercept of each of the regression lines in Figure 3a corresponds to k_{obs} in a buffer-free condition. In a buffer free condition with selected pH values, Equation 8 should be valid, as follows.

$$k_{\text{obs}} = k_{\text{OH}}[\text{OH}^-] + k_{\text{P}}[\text{Total}_{\text{phosphate}}] + k_{\text{A}}[\text{Total}_{\text{ammonia}}] = k_{\text{OH}}[\text{OH}^-] \dots (\text{Equation 8})$$

According to the Equation 8, a plot of k_{obs} against the OH^- ion concentration ought to give a straight line passing through the original $k_{\text{OH}}=0$ (Figure 3c). The slope of the regression line gave the k_{OH} - value $23.38 \text{ l mol}^{-1} \text{ min}^{-1}$

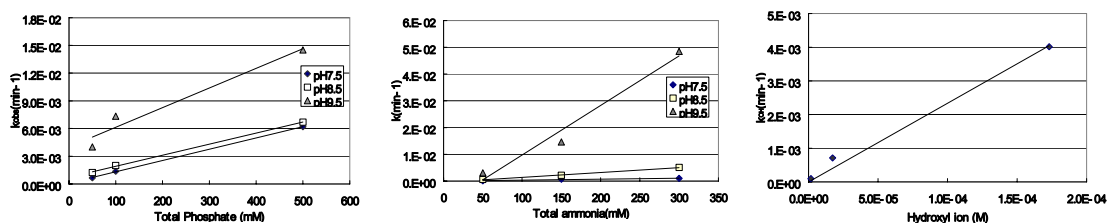


Figure 3a, b & c. From left to right: Effects of phosphate, ammonia and hydroxyl ion.

Comparison of the experimental result with simulated one

The reduction profiles of amoxicillin in SD1 and SD2 and the calculated degradation profiles using the data in Table 1 are shown in Figure 4. Since the calculated reduction profiles showed good agreement with the experimental data, the decay of amoxicillin in the toilet matrix seemed to be mainly subject to the concentrations of phosphate, ammonia and pH.

Table 1. Concentration of phosphate, ammonia and pH in the sawdust matrix.

	SD 1	SD 2
pH	7.4	8.8
$[\text{Total}_{\text{phosphate}}]$	460	460
$k_{\text{H}_2\text{PO}_4^-} f_{\text{H}_2\text{PO}_4^-}$	4.39×10^{-6}	2.72×10^{-7}
$k_{\text{HPO}_4^{2-}} f_{\text{HPO}_4^{2-}}$	7.57×10^{-6}	1.20×10^{-5}
$[\text{Total}_{\text{ammonia}}]$	6	190
$k_{\text{NH}_3} f_{\text{NH}_3}$	2.52×10^{-6}	2.41×10^{-5}
$k_{\text{NH}_4^+} f_{\text{NH}_4^+}$	3.95×10^{-7}	1.50×10^{-7}
$k_{\text{OH}}[\text{OH}^-]$	3.21×10^{-5}	8.07×10^{-4}

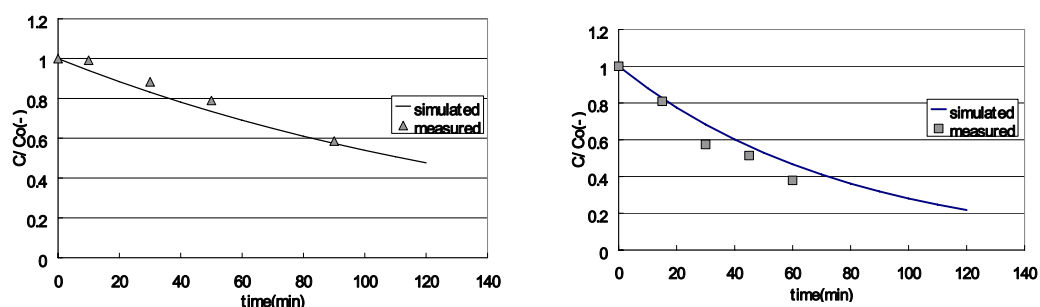


Figure 4. Comparison between the experimental (SD1 left, SD2 right) and simulated result.

Estimation of amoxicillin reduction rate in the composting toilet

Figure 5 shows the changes to the concentration of phosphate, ammonia and pH in the sawdust matrix, and we estimated the amoxicillin degradation rate in the demonstration plant from these data. As Figure 5 reveals, the estimated degradation rate of amoxicillin was strongly related to the ammonia concentration in the demonstration plant. Since phosphate and ammonia accumulate in the composting toilet matrix, we can expect that the more the toilet is used, the faster will be the reduction of amoxicillin.

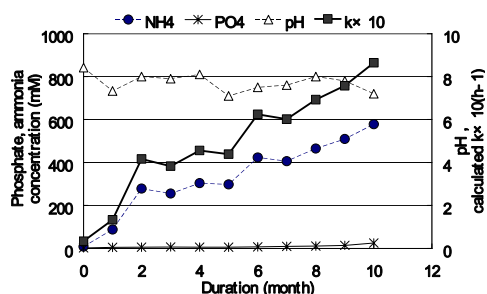


Figure 5. Concentration of phosphate and ammonia, and pH in the sawdust matrix.

CONCLUSIONS

In this study, we investigated the factors affecting the decline of amoxicillin in the composting toilet from the view point of both their biological and non-biological aspects. Our results indicated that amoxicillin was reduced mainly by non-biological factors (phosphate ammonia and pH level in the toilet matrix). The simulation model developed in this study is capable of describing the experimental amoxicillin reduction profiles in sawdust matrix, and since the concentrations of non-biological factors in the toilet matrix increased according to the duration of operation in the demonstration plant,



it will be easier to treat amoxicillin if the composting toilet matrix operates on human excrement over a long period of time.

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REFERENCES

1. Lopez, Zavala, M., A., Funamizu, N., Takakuwa, T. *Onsite Wastewater Differentiable Treatment System: Modeling Approach*. Water Sci. Technol. 46, 317-324. 2002.
2. Jones, O., A., H., Voulvoulis, N., Lester, J., N. *Aquatic environmental assessment of the top 25 English prescription pharmaceuticals*. Water Res. 36, 5013-5022. 2002.
3. Khan, S., J., Ongerth, J., E. *Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations*. Chemosphere. 55, 355-367. 2004.
4. Kakimoto, T., Osawa, T, Funamizu, N. *Antibiotic effect of amoxicillin in human excrement on the feces composting process, and reactivation of bacteria by intermittent feeding of feces*. Bioresource Technology. (submitted)
5. Goodman & Gillman. *Goodman & Gillman's the pharmacological basis of therapeutics*. Tenth edition: pp.1189-1218. McGraw-Hill. 2001.
6. Trevors, J., T. *Sterilization and inhibition of microbial activity in soil*. J. Microbiol. Meth. 26, 53-59. 1996.
7. Lopez, Zavala, M., A., Funamizu, N., Takakuwa, T. *Temperature effect on aerobic biodegradation of feces using sawdust as a matrix*. Water Res. 38,2406-2416. 2004.
8. Lopez, Zavala, M., A., Funamizu, N. *Effect of Moisture on the Composting Process in the Bio-Toilet System*. Compost Sci. Util. 13, 208-216. 2005.
9. Brid, A., E. *Analytical profiles of drug substances*. 23, 1-52. 1994.
10. Bundgaard, H. *Polymerization of penicillins II. Kinetics and mechanism of dimerization and self-catalyzed hydrolysis of amoxicillin in aqueous solution*. Acta Pharm. Suec. 14, 47-66. 1977.
11. Zia, H., Shalchian, N., Borhanian, F. *Kinetics of Amoxicillin Degradation in Aqueous Solutions*. Can. J. Pharm. Sci. 12, 3 80-83. 1977.
12. Smith, R., W., Popp, C., J., Norman, D., I. *The dissociation of oxy-acids at elevated temperatures*. Geochim. Comochim. Act. 50, 137-142. 1986.
13. Nakai et al. *Chemistry handbook*. pp.1053-1054 The Chemical Society of Japan. 1966