DEVELOPMENT OF A MODEL DESCRIBING VIRUS REMOVAL PROCESS IN AN ACTIVATED SLUDGE BASIN

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The virus removal process from the liquid phase in an activated sludge basin possibly consists of physicochemical processes, such as adsorption onto sludge flocs, biological processes such as microbial predation and inactivation by virucidal components excreted by microbes. To describe properly the virus behavior in an activated sludge basin, a simple model is proposed based on the experimental data obtained using a poliovirus type I. A three-compartment model, which includes the virus in the liquid phase and in the peripheral and inner regions of sludge flocs is employed. By using the model, the virus removal process was successfully simulated to highlight the implication of its distribution in the activated sludge basin.

Introduction

There are many kinds of wastewater which contain not only organic compounds such as BOD, phosphorous and nitrogenous compounds, but also pathogens such as bacteria and enteric viruses¹⁴. Biological treatment processes, especially activated sludge processes, applied to wastewater are effective for reducing the infectivity of pathogens, though their quantitative behavior in the treatment process has been scarcely clarified^{1, 2}. For the advanced design of a process and operation to complete the biological inactivation of these pathogens, it is necessary to reconsider the factors affecting the related phenomena.

Complete virus inactivation by activated sludge has been recognized^{3, 10, 16}, but its mechanism has not been clearly understood. In addition, physical and chemical factors such as pH, temperature and light have also been possible implications on the process^{1, 8}. Most viruses in an activated sludge basin are removed by their adsorption to the sludge flocs^{4, 5}. This removal does not necessarily imply their complete inactivation but their mere transfer from liquid phase to floc surface, where they remain infectious. Some adsorbed viruses subsequently become the prey of microbes, being ultimately inactivated by their virucidal activity^{6, 7}.

This paper's purpose is to extract the dominant factors affecting the virus removal process in an activated sludge basin, and to propose a model describing the process properly to highlight the implication of its elemental kinetics.

1. Materials and Methods

1.1 Activated sludge

The activated sludge used was acclimated for more than six months after having been obtained from a municipal wastewater treatment plant. The acclimation conditions were MLSS (mixed liquor suspended solid) concentrations of $2 \text{ kg} \cdot \text{m}^{-3}$, temperature of 27°C and agitation rate of 200 r.p.m. The sludge was cultured in an aeration tank of 0.01 m^3 working volume by a fill-and-draw mode of 24 hour cycle, where $4 \times 10^{-3} \text{ m}^3$ treated water was withdrawn before synthetic sewage⁸ of the same volume was added.

1.2 Virus type and viral assay

Poliovirus type 1, strain LSc 2ab, was used as a model enteric virus, which was kindly donated by Tokyo Metropolitan Research Laboratory of Public Health. Virus infectivity was assayed as plaque-forming units (PFU) on buffalo green monkey kidney (BGM) cells by using the agar overlay method as previously described⁹. Samples were diluted with Eagle's minimum essential medium supplemented with 1% fetal calf serum.

1.3 Experimental apparatus and procedure

A schematic diagram of the experimental apparatus is shown in **Fig. 1**. The basin was aerated at $2 \times 10^{-4} \text{ m}^3 \cdot \text{min}^{-1}$ through an air sparger installed at the bottom. The $6 \times 10^{-4} \text{ m}^3$ of sludge to be tested was put into a 1 dm^3 beaker, the temperature of which had been controlled at 27°C . The pH was adjusted to 7.0 ± 0.2 .

After the predetermined amount of virus was mixed with the activated sludge, 5 cm^3 of mixed liquor for virus assay was sampled at predetermined intervals. All samples were stored at -70°C until assayed. Samples were separated into sludge solids and supernatants by centrifuging for 15 minutes at 3000 r.p.m. The supernatant was assayed for the residual infectious virus in the liquid phase. The centrifuged

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A : Activated sludge
C : Air pump
E : Temperature controller
B : Water bath
D : Flow meter

Fig. 1 Schematic diagram of experimental apparatus

Fig. 2 Time course of residual virus in the liquid phase of an activated sludge basin

precipitate was assayed for recoverable infectious virus, which had been transferred from the liquid phase, by the following procedure: after 5 minutes elution using 5 cm³ eluate⁹⁾ (a mixed solution of 1M sodium nitrate and 3% beef extract at pH 7.0), the centrifuged supernatant was assayed.

2. Experimental Results

Figure 2 shows examples of the time courses of the residual virus in the liquid phase and the recoverable infectious virus from the filtered MLSS. In the figure, the virus infectivities in the MLSS-free liquid, which had been prepared by filtration using a filter of 0.22 μm pore size, were plotted as a control. The decrease of virus infectivity in the control liquid was negligibly small.

The decrease of residual virus in the liquid phase followed two consecutive first-order processes shown in Eq. (1), i.e., an initial rapidly decreasing period (first stage) and a later slowly decreasing period (second stage).

$$X_t = X_0 e^{-K'_1 t} \quad (1)$$

where, X_0 and X_t are virus concentrations at times 0 and t , respectively, and K'_s ($s=1$ and 2) corresponding to the first and second stages, respectively, is the rate constant.

Fig. 3 Recovery of virus from activated sludge

The rate constants K'_s for the decrease of the poliovirus infectivities in both of the two consecutive stages have been found to be proportional to the MLSS concentration, C_0 , as shown by Eq. (2)⁸⁾.

$$K'_s = \alpha_s \cdot C_0 \quad (2)$$

where α_s ($s=1, 2$) is constant.

The constants α_1 and α_2 were 1.28 m³·kg⁻¹·h⁻¹ and 0.34 m³·kg⁻¹·h⁻¹, respectively. These results suggest that the virus removal mechanisms in the two consecutive steps are different to each other.

Figure 3 shows the time course of the virus elution percentage from the sludge phase. During the first stage, about 70%, at most, of the virus transferred to the sludge phase was recovered by the elution procedure applied in the experiment. The virus unrecoverable throughout the process, i.e., about 30% of the virus transferred to the sludge solid, may be due to a seemingly irreversible adsorption encompassing the inner region of sludge flocs. In the second stage, the virus recovery decreased gradually with contact time. The authors have demonstrated that reduction of the virus recovery efficiency in the second stage was attributed to biological actions such as direct and indirect predations by protozoa and metazoa¹⁵⁾.

To understand the mechanisms of the virus inactivation in the activated sludge basin that will enable proper design of apparatus and operation, it is necessary to clarify the contribution of the elemental mechanisms of the virus removal based on the results and considerations discussed above. For this, a mathematical model which simulates the process properly should be constructed.

3. Modeling, Simulation and Discussion

For necessary and sufficient simplification, the activated sludge mixed liquor is divided into three compartments, which affect the observed virus behaviors, i.e., the liquid phase and the peripheral and inner regions of sludge flocs. The floc peripheral region participates in the reversible virus adsorption process from the liquid

Fig. 4 Schematic diagram of virus transfer in an activated sludge basin

phase. The inner region of the flocs participates in encompassing the adsorbed virus into the inner part of the flocs due to disruption and reformation by continuous aeration and/or agitation. For further simplicity, let the inner floc region include the protozoa and metazoa which directly uptake the virus dispersed in the liquid, and at the same time prey on the virus adsorbed on the floc. These are briefly expressed as "biological actions" in the following discussion. The concept of the compartments is shown in Fig. 4.

For the mathematical description of the process, the following items can be postulated based on the experimental facts described in the previous section.

- The final inactivation of virus can be achieved exclusively in the third compartment (the inner region of sludge flocs).
- The virus in the second compartment (the peripheral region of flocs) is completely recovered by the elution method adopted in this work.
- The virus incorporated into the third compartment is not recovered by the elution method.

Let the amount balance equation for the virus in the activated sludge basin of working volume V be considered as,

$$V \cdot X_0 = V \cdot X_L + V \cdot X_E + V \cdot X_I \quad (3)$$

where X_0 is the initial virus concentration in the basin, and X_L , X_E and X_I are the virus concentrations in the liquid (the first compartment) and in the peripheral and inner regions of flocs (the second and third compartments), respectively. These concentrations are based on the mixed liquor volume V . This is because of the difficulty in determining the volumes of the peripheral and inner floc regions.

Considering the virus behavior shown in Fig. 2, linear kinetics for the changes in the virus concentrations are assumed as follows:

$$dX_L / dt = -K_1 X_L + K_2 X_E - K_5 X_L \quad (4)$$

$$dX_E / dt = K_1 X_L - K_2 X_E - K_3 X_E + K_4 X_I \quad (5)$$

$$dX_I / dt = K_5 X_L + K_3 X_E - K_4 X_I \quad (6)$$

where K_1 , K_2 , K_3 , K_4 and K_5 are rate constants.

Eqs. (4), (5) and (6) represent the variations of residual virus in the liquid phase and virus in the peripheral and inner regions of sludge flocs, respectively.

For a short contact time, MLSS concentration C_0 and the volumes of compartments 2 and 3 can be assumed constant.

$$C_b = \epsilon C_0, C_p = (1 - \epsilon) C_0 \quad (7)$$

where C_b and C_p are MLSS concentrations of the peripheral and inner regions of sludge flocs, respectively, and ϵ is the volume fraction of the peripheral region of sludge flocs.

Based on the facts expressed by Eq. (2), it is assumed that all the rate constants K_j ($j=1, 2, 3, 4, 5$) in Eqs. (4), (5) and (6) are proportional to MLSS concentration C_0 ¹⁵. Eqs. (4), (5) and (6) are rewritten as

$$dX_L / dt = -k_1 C_0 X_L + k_2 C_0 X_E - k_5 C_0 X_L \quad (8)$$

$$dX_E / dt = k_1 C_0 X_L - k_2 C_0 X_E - k_3 C_0 X_E + k_4 C_0 X_I \quad (9)$$

$$dX_I / dt = k_5 C_0 X_L + k_3 C_0 X_E - k_4 C_0 X_I \quad (10)$$

where $k_1 = \epsilon k_1'$, $k_2 = \epsilon k_2'$, $k_3 = (1 - \epsilon) k_3'$, $k_4 = (1 - \epsilon) k_4'$ and $k_5 = (1 - \epsilon) k_5'$.

The simultaneous first-order equations, Eqs. (8), (9) and (10), can be solved directly by a numerical method¹⁷ on a microcomputer under the following initial conditions.

$$\begin{aligned} X_L &= X_0 \\ X_E &= 0 \\ X_I &= 0 \end{aligned} \quad \text{at} \quad t = 0 \quad (11)$$

Figure 5 (a), (b) and (c) show examples of comparisons between the simulated and experimental results corresponding to MLSS concentrations of 2, 3 and 4 $\text{kg} \cdot \text{m}^{-3}$, respectively. The simulated residual virus amounts in the liquid phase (VX_L) and recovered from the sludge phase (VX_E) agree reasonably well with the experimental results.

Figure 6 shows comparisons of the simulated and experimental residual virus amount in the liquid phase, VX_L , on a semilogarithmic scale, which explains the two consecutive first-order processes very well. From these results, the proposed model is proven to be able to describe the virus removal process in the activated sludge basin.

This model can also predict the change in virus concentration in the inner region, X_I , as shown in Fig. 5. Although the amount of virus in the inner region, VX_I ,

Fig. 5 Comparison of calculated and experimental results

seems to be inactivated, it includes viruses irrecoverable by the biological actions and encapsulated within the sludge flocs. It provides a better understanding of the mechanisms of virus removal by activated sludge. For example, X_L and X_E decreased gradually after 0.5-1 hour (the second stage) while X_I increased. This implies that virus removal in the second stage occurs by the irreversible removal mentioned above. It was also shown that viruses in the liquid phase decreased rapidly between 0-0.5 hour (the first stage),

Fig. 6 Semi-logarithmic plot of the residual virus

accompanying a considerable amount of virus transfer to the peripheral region of the sludge flocs and some transfer to the inner region. Therefore, it can be safely concluded that the removal of viruses in the first stage occurred primarily by adsorption onto the sludge flocs.

The virus removed from the liquid phase after 5 hours reached 90% of the initial infectious virus irrespective of the MLSS concentration. However, much of the recoverable virus was observed in the peripheral region of the sludge phase. This implies that some of the adsorbed virus may be released to the liquid phase by the disruption of flocs or changes in physicochemical and biological conditions during the treatment. To obtain well treated effluents, therefore, the activated sludge should maintain excellent viral adsorption ability and the process should separate the sludge completely.

To consider more precisely virus removal by activated sludge, the kinetic constants at the first and second stages obtained from data fitting by simulation were plotted in Fig. 7. These constants are affected by factors such as the characteristics of sludge flocs and virus peripheral region, and the activity of microbes composing the sludge flocs.

In the first stage, the rate constant k_1 relating to adsorption to the peripheral region of sludge flocs was much larger than k_3 and k_5 which are related to microbial predation, virus entrapment into the inner floc region and microbial virucidal effect. In the second stage, k_5 became relatively large compared with k_1 . These results are interpreted by the fact that the virus removal process followed two consecutive first-order processes shown in Fig. 2. Namely, virus removal in the first stage depends largely on reversible adsorption, while in the second stage it depends on biological action. It is also shown that the values of k_2 and k_4 , particularly in the second stage, decreased with an increase of MLSS concentration. This may be due to a possible decrease in the frequency of disruption and reconstruction of sludge flocs with increase of MLSS concentration.

Figure 8 (a), (b) and (c) show simulation examples of the virus behavior under particular conditions. Fig. 8(a)

a) 1st stage

b) 2nd stage

Fig. 7 Relation between rate constants and MLSS concentration for the 1st stage and the 2nd stage
(Key: $\square$, k_1 ; $\blacklozenge$, k_2 ; $\bullet$, k_3 ; $\triangle$, k_4 ; $\blacksquare$, k_5)

is the case where k_1 is much greater than k_3 and k_5 , i.e., the virus in the liquid phase is removed dominantly by adsorption onto floc peripheral regions. Fig. 8 (b) describes the case where k_3 and k_5 are greater than k_1 , i.e., the virus is removed by biological action. In Fig. 8 (c), the case where both adsorption and biological action are effective for removal is shown. These results imply that high MLSS concentration, high adsorption rate of virus onto sludge peripheral and high activity of microbes are important for virus removal from the liquid phase.

For practical application of the proposed model, the above mentioned factors, which have been interpreted by the kinetic constants, can be exploited as indices for monitoring the treatment process, anticipating the attainable level of virus removal during a given operational period. Furthermore, the proposed model is applicable to a continuous culture because the behavior and removal mechanism of virus in the batch culture are essentially similar to those in the continuous culture. It is considered that the contact time in the present analysis corresponds to the hydraulic retention time in a continuous culture. However, in order to develop a more comprehensive model for a continuous

Fig. 8 Examples of the simulation

system, further study is needed.

Conclusion

Virus removal by activated sludge occurs by adsorption to the peripheral region of sludge flocs, entrapment in the inner region of sludge flocs, microbial predation and possible microbial virucidal components.

The decrease of viral infectivity in a liquid phase consisting of two consecutive first order processes, i.e., a rapidly decreasing first stage and a slowly decreasing second stage, was simulated successfully by a three-compartment model. The model successfully interprets the amount of residual viruses in the liquid phase, the amount of recoverable viruses in the sludge phase and the amount of apparently inactivated virus.

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Nomenclature

C_0	= MLSS concentration in the reactor	[kg·m ⁻³]
C_b	= MLSS concentration of the peripheral region of sludge flocs	[kg·m ⁻³]
C_p	= MLSS concentration of the inner region of sludge flocs	[kg·m ⁻³]

K_s' (1 or 2)	= observed rate constant in the first stage and the second stage	[h ⁻¹]
K_1, K_2, K_3, K_4, K_5	= rate constant	[h ⁻¹]
k_1, k_2, k_3, k_4, k_5	= rate constant	[m ³ ·kg ⁻¹ ·h ⁻¹]
$k_1', k_2', k_3', k_4', k_5'$	= rate constant	[m ³ ·kg ⁻¹ ·h ⁻¹]
t	= contact time	[h]
X_0	= initial virus concentration	[PFU·cm ⁻³]
X_E	= virus concentration in the peripheral region of sludge flocs	[PFU·cm ⁻³]
X_i	= virus concentration in the inner region of sludge flocs	[PFU·cm ⁻³]
X_L	= virus concentration in the liquid phase	[PFU·cm ⁻³]
V	= volume of mixed liquid	[m ³]
α_1	= rate constant in the first stage	[m ³ ·kg ⁻¹ ·h ⁻¹]
α_2	= rate constant in the second stage	[m ³ ·kg ⁻¹ ·h ⁻¹]
f	= volume fraction of the peripheral region of sludge flocs	[-]

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