Effect of Activated Sludge Process and Sludge Storage on Removal of *Escherichia coli* O157:H7

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Abstract

An experimental investigation was carried out on removal characteristics of Verotoxin-free *Escherichia coli* O157:H7 in a batch culture of activated sludge and stored sludge, and then its profiles in a domestic wastewater treatment facility was discussed.

Domestic wastewater containing about 10^4 CFU/ml of *E. coli* O157:H7 was treated by activated sludge (MLSS concentration was about 2,500 mg/l) at 25°C. *E. coli* O157:H7 was almost completely removed from the supernatant after 8 hrs, and fecal coliforms and total coliforms were reduced considerably. Although the bacteria in the activated sludge mixed liquor decreased with aeration time, *E. coli* O157:H7 still survived after 24 hrs. *E. coli* O157:H7 was almost all removed from the stored sludge after 30 days while for fecal coliforms and total coliforms it took up to 150 days for removal.

We proposed a case study model for *E. coli* O157:H7 removal in a middle-scale domestic wastewater treatment facility using the extended aeration process where the bacterial contamination occurs at the level of $10^4 \sim 10^5$ CFU/ml. The number of *E. coli* O157:H7 bacteria declines in the aeration tank during the 24 hr aeration period and is almost completely removed from the resulting biologically treated water. If 0.5% of influent is taken from the sedimentation tank as excess sludge, *E. coli* O157:H7 survives in this sludge at the level of $10^2 \sim 10^3$ CFU/ml. The bacteria in the sedimentary sludge increases by thickening but is almost completely removed during storage over a month.

Key words : *Escherichia coli* O157:H7, coliforms, domestic wastewater, activated sludge, sludge storage

INTRODUCTION

Escherichia coli responsible for diarrhea is classified into Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, Enteroinvasive *E. coli* and Enterohemorrhagic *E. coli*. Pathogenic *E. coli* O157:H7, by which food poisoning has often occurred in Japan, resulting in an increasing number of deaths¹⁾, belongs to Enterohemorrhagic *E. coli* and is taxonomically expressed "*E. coli* O157:H7,

VT(+)" because of producing Verotoxin of a strong toxicity.

In Japan, an outbreak of food poisoning by pathogenic *E. coli* is immediately followed by preventive measures against the specially designated communicable diseases. However, in most cases, pathogenic *E. coli* excreted from those infected has already entered sewers or domestic wastewater treatment facilities such as Johkasou, before being detected as a pathogen of the food poisoning.

As for the fate of pathogenic microorganisms in wastewater or sludge treatment process, some reports have been made on *Vibrio cholerae*^{2), 3)}, *Salmonella*³⁻⁷⁾ and *Welchii* (*Clostridium perfringens*)³⁾. The behavior or the survival of pathogenic *E. coli* O157:H7 in natural water has been reported⁸⁾⁻¹⁰⁾, but there has been little or no discussion in wastewater treatment process. Therefore, it is very significant to make an experiment to know the removal or the fate of *E. coli* O157:H7 in biological treatment processes and examine the case study model at the domestic wastewater treatment facility which is led from this experiments.

In this paper, an experimental investigation was carried out on removal characteristics of Verotoxin-free *E. coli* O157:H7 in a batch culture of activated sludge and stored sludge, and then its profiles in a domestic wastewater treatment facility was discussed from the obtained results.

MATERIALS AND METHODS

***Escherichia coli* O157:H7** *E. coli* O157:H7, VT(-) strain from Kanto Chemical co. was used in the experiments. This strain was incubated at 36°C for 18 hrs in a TSB (Trypticase Soy Broth) medium (BBL/Difco). A sediment after being centrifuged (4,000 rpm, 10 min) was washed several times in a

phosphate buffer (pH7.2), and was then diluted with this buffer to be approximately 10^6 CFU/ml.

Activated sludge Excess sludge (6,050 mg/l of MLSS) taken from the sludge adjustment and measurement device of a gappei-shori Johkasou, was used in the experiments after being washed by deionized water. This Johkasou was designed for 3,100 users, using the extended aeration process.

Wastewater Domestic wastewater taken from the flow adjustment and measurement device of the above-mentioned Johkasou was used in the experiments, and its BOD concentration was 240 mg/l.

Experimental apparatus The schematic diagram of experimental apparatus is shown in Fig. 1, composed of a 1l glass reactor. The air flowing was supplied at 0.5l/min to the reactor. The waste air through a sterile filter (0.2 μ m) was flowed to disinfectant solution. The reactor was placed in a constant-temperature room (25°C).

Methods Four hundreds ml of wastewater was added in the reactor. A fixed volume of *E. coli* O157:H7 or the activated sludge was inoculated into the wastewater in each Run, and then the wastewater was aerated for 24 hrs. Inoculum of RUN 1 was activated sludge, that of RUN 3 was *E. coli* O157:H7, and that of RUN 2 was both. Initial concentration of *E. coli* O157:H7 was set up about 10^4 CFU/ml. This concentration was decided from reference literature^{2), 8)}.

And initial conditions of activated sludge were 3,000 mg/l as MLSS in each experiment. During the aeration, about 50ml of the activated sludge mixed liquor in the reactor was periodically taken for analysis. The bacteria were counted in each mixed liquor and in the supernatant obtained after settlement of the mixed liquor.

After the 24 hr aeration period, the activated sludge mixed liquor of RUN 1 and RUN 2 was left for about 4 hrs and the settled sludge was obtained by discarding the supernatant. The settled sludge, moved to capped 200ml bottles, was left in anaerobic condition at 25 °C for 150 days. As for Run 3, the liquor was stored in anoxic condition at 25°C. During this storage, the bacteria in the sludge (RUN 1 and RUN 2) or in the

Fig. 1 Experimental apparatus.

liquor(RUN 3) were counted periodically.

Analysis *E. coli* O157:H7 was measured with a CHROMagar O157 medium (CHROMagar co.)⁽¹¹⁾, being incubated at 42°C for 24 hrs. Fecal and total coliforms were counted at 36°C for 24 hrs using a Chromocult Coliform Agar medium (Merck co.)⁽¹²⁾. The standard plate count bacteria were measured using a standard agar culture medium (Nissui pharm. co.) at the same condition as fecal and total coliforms.

RESULTS

Water quality and microscopic observation

Although the BOD of the original domestic wastewater was 240 mg/l, that of the supernatant for RUN1 and RUN2 decreased to 7~10mg/l after a 24 hr treatment with the activated sludge. But for Run 3, the BOD was 190mg/l and showed little decrease, since the activated sludge had not been added. The MLSS concentration of the freshly-prepared mixed liquor for RUN1 and RUN2 ranged from 2,550 to 2,700 mg/l, and that of the mixed liquor after 24 hr aeration was almost the same. The thickened sludge, obtained by settling the mixed liquor and discarding the supernatant after the aeration experiment, was 7,650 ~7,800 mg/l of MLSS. So, the mixed liquor was thickened about 3 times. The pH of the thickened sludge for RUN 1 and RUN2 was 6.9 ~7.0 and then changed to 5.8~6.1 after 150 day storage. The ignition loss of thickened sludge for RUN 1 and RUN 2 was 64.5 ~67.7% and then changed to 57.6 ~58.6% after the storage.

Ciliata, Sarcodina, and Aschelminthes were microscopically observed. Namely, *Epistylis* sp. was by 20~25 N/ml, *Aspidisca costata* was by 200~220 N/ml, *Litonotus* sp. was by 10~15 N/ml, *Spirostomum* sp. was by 10~12 N/ml, *Amoeba* sp. was by 15~18 N/ml, *Arcella* sp. was by 30~36 N/ml, and *Philodina* sp. was by 15~18 N/ml, respectively. The number of these microorganisms did not change much during the aeration. *Vorticella microstoma* and *Chaetonotus* sp. which had inhabited the aeration tank of Johkasou were not detected in this experiment.

Fate of bacteria Table 1 shows the survival of the bacteria in the supernatant

obtained after settlement of the mixed liquor. *E. coli* O157:H7 was almost completely removed on RUN 2 after an 8 hr aeration with the activated sludge, but definite decline was not observed on RUN 3 without the activated sludge even after a 24 hr aeration period. Similarly, fecal coliforms and total coliforms decreased with aeration time on RUN 1 and RUN 2 where the activated sludge was added, but they did not decrease so much on RUN 3.

Table 2 shows the survival of the bacteria in the activated sludge mixed liquor on RUN 1 and RUN 2. Although a decline with time was observed for each bacteria, 7.5×10^2 CFU/ml of *E. coli* O157:H7, $0.7 \sim 1.2 \times 10^3$ CFU/ml of fecal coliforms, and $6.5 \sim 9.2 \times 10^3$ CFU/ml of total coliforms still survived even after a 24 hr aeration period.

Table 3 shows the survival of the bacteria over a 150 day period in the stored sludge following the 24 hr aeration process. *Escherichia coli* O157:H7 whose initial concentration was 1.4×10^3 CFU/ml was almost completely removed after 30 days on RUN 2, and 3.4×10^4 CFU/ml of *E. coli* O157:H7 was almost completely removed after 60 days on RUN 3. Similarly, fecal coliforms and total coliforms which still survived after 90 days were removed after 150 days for all three test cases. Standard plate count bacteria, whose initial concentrations on RUN 1 and RUN 2 were at the level of 10^7 CFU/ml, decreased to 10^6 CFU/ml, while for RUN 3, the initial level of 10^7 CFU/ml decreased to 10^4 CFU/ml after 150 day storage.

DISCUSSION

Removal of *E. coli* O157:H7 by activated sludge

Experiments were carried out on the assumption that about 10^4 CFU/ml of *E. coli* O157:H7 survived in an aeration tank. *E. coli* O157:H7 was removed after more than 8 hr aeration with 2,500 mg/l of MLSS. Figure 2 shows the relationship between aeration time and survival ratio of bacteria for RUN 2. Each bacterium in the supernatant did not grow but decreased rapidly with aeration time. Especially the survival ratio of *E. coli* O157:H7 decreased to less than 0.1 % after 8 hrs, that is, more than 99.9% of *E. coli* O157:H7 was removed. Fecal coliforms and total coliforms decreased at a rate similar to

Table 1 Survival of the bacteria in the supernatant

Time (hrs)	Counts (CFU/ml)			
	0	3	8	24
<i>E. coli</i> O157:H7				
RUN 1	N.D.	N.D.	N.D.	N.D.
2	1.2×10^4	4.0×10^2	<10	<10
3	6.8×10^4	4.9×10^4	—	4.4×10^4
Fecal coliforms				
RUN 1	1.4×10^4	6.6×10^2	9.5×10	3.6×10
2	3.7×10^4	8.5×10^2	8.0×10	1.0×10
3	1.1×10^5	1.0×10^5	—	7.8×10^4
Total coliforms				
RUN 1	5.6×10^4	3.3×10^3	6.3×10	1.4×10^2
2	7.8×10^4	5.2×10^3	3.2×10^2	4.2×10
3	2.2×10^5	2.3×10^5	—	2.1×10^5

N.D. : not detected
— : not tested

Table 2 Survival of the bacteria in the activated sludge mixed liquor

Time (hrs)	Counts (CFU/ml)			
	0	3	8	24
<i>E. coli</i> O157:H7				
RUN 1	N.D.	N.D.	N.D.	N.D.
2	6.4×10^4	—	4.5×10^3	7.5×10^2
Fecal coliforms				
RUN 1	1.8×10^4	—	—	0.7×10^3
2	8.4×10^4	—	7.2×10^3	1.2×10^3
Total coliforms				
RUN 1	8.0×10^4	—	—	6.5×10^3
2	1.5×10^5	—	1.4×10^4	9.2×10^3

N.D. : not detected
— : not tested

Table 3 Survival of the bacteria in the stored sludge

Time (days)	Counts (CFU/ml)								
	0	1	4	14	30	60	70	90	150
<i>E. coli</i> O157:H7									
RUN 1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2	1.4×10^3	7.5×10^2	1.6×10^2	5.5×10	<10	<10	<10	<10	<10
3	3.4×10^4	3.0×10^4	1.4×10^4	1.8×10^3	9.5×10	<10	<10	<10	<10
Fecal coliforms									
RUN 1	5.0×10^3	3.0×10^3	1.8×10^3	1.3×10^3	4.5×10^2	3.0×10^2	2.5×10^2	6.0×10	<10
2	3.0×10^3	2.8×10^3	2.1×10^3	8.2×10^2	1.5×10^2	5.8×10	2.0×10	2.0×10	<10
3	7.8×10^4	4.1×10^4	2.5×10^4	6.4×10^3	3.5×10^2	6.0×10	4.0×10	2.5×10	<10
Total coliforms									
RUN 1	2.0×10^4	1.6×10^4	8.2×10^3	6.2×10^3	2.5×10^3	4.4×10^2	3.7×10^2	7.0×10	<10
2	2.8×10^4	2.2×10^4	1.2×10^4	4.2×10^3	5.8×10^2	1.8×10^2	4.0×10	3.0×10	<10
3	2.1×10^5	1.3×10^5	6.5×10^4	3.1×10^4	9.6×10^2	2.3×10^2	1.3×10^2	5.0×10	<10

N.D. : not detected

E. coli O157:H7, which suggests that coliforms may give a similar decline curve.

From Table 2, by comparing the bacteria concentrations about RUN 1, which was composed with domestic wastewater and sludge, and RUN 2, which added *E. coli* O157 bacteria solution to them, we calculated the proportions of the fecal coliforms and total coliforms that originated from the added *E. coli* O157 bacteria. And at the beginning of the preparation, they were 78% and 47%, respectively. But after a 24 hr aeration they decreased to 42% and 29%. In other words, the proportions of fecal coliform and total coliform of *E. coli* O157 bacteria origin

became low as the aeration went on. This showed that there was the trend that coliforms of domestic wastewater and sludge origin were difficult to decrease than *E. coli* O157 bacteria origin.

It has been reported that pathogenic bacteria such as *Vibrio cholerae*³⁾ and *Salmonella*^{3), 4), 6)} in wastewater are removed by biological treatment in parallel with a high BOD removal efficiency. Curds *et al.* studied on some kinds of ciliated protozoa feeding upon bacteria¹³⁾ and proved the removal of *E. coli*¹⁴⁾ by protozoa in the activated sludge process. And they explained that predation by protozoa such as *Epistylis* sp. and *Aspidisca*

sp. inhabiting the activated sludge was most important. Also, Yaziz *et al.*⁶⁾ proposed the model of the removal of *Salmonella enteritidis* in activated sludge. They supported the theory of predation by ciliated protozoa, and demonstrated the protozoa played a major role in removing bacteria from the mixed liquor. In our experiments, these mechanisms may probably relate to *E. coli* O157:H7 removal by activated sludge. The scale of domestic wastewater treatment systems in Japan ranges from gappei-shori Johkasous designed for 5 users to sewage treatment facilities designed for tens of thousands of users. The initial concentration of *E. coli* O157:H7, 10^4 CFU/ml, was determined on the assumption that it entered a small-scale facility. Therefore, at large-scale facilities, where the concentrations of bacteria in the reactors are low because of the dilution with wastewater, the removal may require less time than our results. The results of this experiment suggest that biological treatment can almost completely remove the bacteria at most facilities. Generally, the aeration time should be more than 8 hrs for conventional

activated sludge process or more than 16 hrs for extended aeration processes. Biologically treated water is disinfected with chlorine before discharge. Therefore *E. coli* O157 in water-borne need hardly be worried about if residual chlorine can be detected in the effluent.

On the other hand, Fig. 2 demonstrates *E. coli* O157:H7 still survived in the activated sludge mixed liquor after a 24 hr aeration period at a survival ratio of more than 1% of the bacteria. In conventional wastewater treatment facilities, the sedimentary sludge in a clarifier or sedimentation tank is continuously returned to biological reactors. Therefore, once contamination by *E. coli* O157:H7 breaks out, it will last for a long time in the biological reactor, and $10^2 \sim 10^3$ CFU/ml of *E. coli* O157:H7 (see Table 2, 3) will be left in the excess sludge.

Effect of sludge storage

Figure 3 shows the survival ratio of bacteria in the stored sludge. The existence of each bacteria declined with time. The survival ratio of *E. coli* O157:H7 decreased to 1 % in about 30 days and was stable thereafter. Fecal coliforms and total coliforms required 150 days to be almost completely removed.

Fig. 2 The survival ratio of bacteria in the activated sludge system (RUN 2).

Symbols : ○, *E. coli* O157:H7;
 △, fecal coliforms;
 ◇, total coliforms;
 ●, *E. coli* O157:H7;
 ▲, fecal coliforms;
 ◆, total coliforms

Fig. 3 The survival ratio of bacteria in the stored sludge (RUN 2).

Symbols : ●, *E. coli* O157:H7;
 △, fecal coliforms;
 ◇, total coliforms

As described above, it was proved that *E. coli* O157:H7 and coliform bacteria were able to survive in the sludge for a long time. The putrefaction, being acidified the sludge during more than a month's storage in anoxic condition, might be one of the reasons for the steady decline of the bacteria. It has been reported that *V. cholerae* survived for 13 days at 30°C after being added to excess sludge at the level of 10^4 CFU/ml²⁾, and that *Salmonella* sp. and fecal coliforms survived at a high level in the stored sewage sludge after a year⁶⁾. These examples can not easily be compared with our results because of differences in most suitable conditions for each bacterium. It may be estimated, however, that pathogenic microorganisms survive for considerably long time in sludge containing a high concentration of organic matter^{15), 16)}. This suggests that the treatment of excess sludge needs aerobic thermophilic digestion¹⁷⁾ or composting¹⁸⁾.

Escherichia coli O157:H7 profiles in domestic wastewater treatment facility

Figure 4 shows the case study which is led from this experiments. We set up the model if *E. coli* O157:H7 contamination occurs in the domestic wastewater at the level of $10^4 \sim 10^5$ CFU/ml and enters into a treatment facility using the extended aeration process. In the aeration tank, where MLSS is about 3,000

mg/l, 200 mg/l of BOD in the influent will be removed efficiently and decrease to under 10 mg/l in the effluent after the 24 hr aeration period. Along with that, the number of *E. coli* O157:H7 will also decrease and may be under 10 CFU/ml in the effluent. And *E. coli* O157:H7 in the excess sludge which is 0.5 % volume of influent will survive at the level of $10^2 \sim 10^3$ CFU/ml. In the beginning the bacteria levels at the sludge thickening or storage tank will increase a little by thickening. But one month later *E. coli* O157:H7 in the storage sludge will be almost completely removed.

CONCLUSIONS

Domestic wastewater containing about 10^4 CFU/ml of *E. coli* O157:H7 was treated with activated sludge (MLSS was 2,500 mg/l) at 25°C. Consequently, *E. coli* O157:H7 was almost completely removed from the supernatant after an 8 hr aeration, and fecal coliforms and total coliforms were reduced considerably. Although the bacteria in the activated sludge mixed liquor decreased with aeration time, *E. coli* O157:H7 still survived after 24 hrs. *Escherichia coli* O157:H7 was almost completely removed from the stored sludge after 30 days while for fecal coliforms and total coliforms it took up to 150 days.

Note: unit for bacteria is CFU/mL
figures in parentheses show percentage of quantity

Fig. 4 A model for *E. coli* O157:H7 removal in domestic wastewater treatment facilities using aeration process.

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