Fate of Coliphage in a Wastewater Treatment Process

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The fate of coliphage in a wastewater treatment plant in the central part of Japan was investigated from March to December 2001. A relative abundance of coliphage, 1000–10,000 PFU/ml determined with three different Escherichia coli strains, was detected in the influent. But, no remarkable seasonal change in the phage concentration in the influent was observed during the ten-month test period. Almost ten times higher coliphage concentration was detected by the F+ E. coli strain than by the other two F– strains. The RNA phage was more stable than the DNA phage against aerobic treatment using activated sludge. Most of the phages in the influent and primary settling tank were detected as suspended forms. Anaerobic-aerobic treatment enhanced adsorption of the phage by the solid particles. Almost no phage was detected in the effluent. Aerobic treatment using activated sludge and/or the addition of flocculants such as PAC was effective for the removal of coliphage, an index of enteric viral pollution.

[Key words: Escherichia coli, domestic wastewater, wastewater treatment process, coliphage, activated sludge]

Domestic wastewater has been documented as a vector of viral diseases, but many of the primarily involved agents are difficult to detect. For practical purposes such as the monitoring of wastewater treatment processes and formulation of quality standards for water effluent, a model indicator would be desirable. Bacteriophage has the potential for use as a process indicator of viruses (1–3). The physical structure of certain types of bacteriophage resembles that of enteroviruses and such types can be cultivated by a simple, rapid, accurate and inexpensive method. Considerable attention has been paid to bacteriophages capable of infecting Escherichia coli because of its specific fecal nature. The detection of somatic coliphage, which binds to receptors located on the cell wall (somatic receptors), varies with the host strain. E. coli C is the most productive host for somatic coliphage (4). Alternatively, F-specific bacteriophages have been proposed as suitable model indicators of the concentration of human enteric viruses in a water treatment process because of their size and relatively high resistance to inactivation (5).

Activated-sludge treatment is known to reduce the concentration of enteric pathogens. It was suggested that the loss of virus during this treatment is due to the attachment of viruses to wastewater particulates, which subsequently settle and become a component of sludge (6). Other evidence indicates that mixed liquor suspended solids (MLSS) of activated sludge inactivate and remove virucidal agents (7). Most studies of bacteriophages in a wastewater treatment process are based on the characterization of pure phages seeded to the process or model environments. There are marked differences between the removal and/or inactivation of seeded and naturally occurring bacteriophages. In this study, the seasonal change in the coliphage concentration was determined using three different E. coli host strains in the influent of a domestic wastewater treatment plant and the fate of phages during the processing was monitored for ten months.

Samples for phage enumeration were obtained from an urban wastewater treatment plant in the central part of Japan, approximately monthly from March to December 2001. A schematic diagram, indicating ports for sampling, is shown in Fig. 1. The number of inhabitants around the plant was about 200,000. Most of the contamination was of human origin. No animal farms or industries were located nearby. The daily volume of the influent was 28,000 m³.

![Fig. 1.](image)

(A) Schematic diagram indicating ports for sampling. Numbers in the diagram indicate the position of the sampling ports: 1, influent; 2, supernatant from the primary settling tank; 3, anaerobic tank; 4, aerobic tank; 5, returned sludge; 6, supernatant from the final settling tank; and 7, effluent. (B) Averages of solid content.

<table>
<thead>
<tr>
<th>Sampling port</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid content (mg wet weight m⁻³)</td>
<td>3.4</td>
<td>1.4</td>
<td>39.6</td>
<td>44.1</td>
<td>91.7</td>
<td>1.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

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There was no rainwater inflow. Hydraulic retention times at each step were: 55 min in the primary settling tank, 60 min in the anaerobic tank, 6 h in the aerobic tank, and 4 h in the secondary settling tank. Polyaluminum chloride (PAC), at a final concentration of 2.5 mg-Al/l, was added to the secondary settling tank as a flocculant. Sodium hypochlorite (NaClO) was added to the chlorination tank to a final concentration of 0.02 mg-free-Cu/l. The average parameter values for influent quality for a period of one year were BOD, 260 mg/l; COD, 120 mg/l; and total suspended solids (TSS), 190 mg/l; and the average parameter values for effluent quality were: BOD, 3 mg/l; COD, 10 mg/l; and TSS, 5 mg/l. Samples were pooled in sterile 100 ml tubes at 4°C and transported to the laboratory within 24 h. An aliquot (50 ml) of each sample was centrifuged (1000g, 10 min), and then the supernatant was carefully transferred to a new sterilized tube, centrifuged (13,000g, 5 min) and subjected to the enumeration of suspended phages. The solid content of a sample was defined as the wet pellet concentration in mg (wet weight)/g after the centrifugation at 1000g, indicating that the proportion of RNA phage was 1/3 compared to the value obtained without RNase treatment, indicating that the proportion of RNA phage was 1/3.

The phage concentration at each step of the wastewater treatment process was determined to monitor the fate of the phages. The arithmetical mean of the phage concentration during the test period is shown in Fig. 3. General trends in the fate of phages each month were almost identical. Since the phage concentration in the effluents from the secondary settling tank and the chlorination vessel dropped below the identification limit for the plaque assay in some cases, the arithmetical mean instead of the geometrical mean was used for the analysis. The concentrations of phages infectious to E. coli K-12 (W3110) F<sup>+</sup>, E. coli HfrH (IAM 12017) F<sup>+</sup>, and E. coli C remained almost constant in the influent and primary settling tank. A slight decrease was observed in the anaerobic tank. On the other hand, a significant decrease in the phage concentration was observed in the aerobic tank and in the secondary settling tank. Only a few phages (PFU/ml) were detected in the supernatant of the secondary settling tank. RNase treatment of the sample from the aerobic tank compared to the value obtained without RNase treatment, indicating that the proportion of RNA phage was 1/3–1/2.

Coliphage infectious to the three E. coli strains was constitutively detected in the supernatant of the influent from March to December 2001. It was reported that E. coli C was generally found to yield the highest plaque counts among E. coli species (8). This can be explained by the absence of a DNA restriction system in this strain and by the presence of a broad range of receptors. However, the most abundant phage detected in the influent in this study was that infectious to E. coli HfrH (IAM 12017) F<sup>+</sup>, with a value of 1x10<sup>5</sup>–1x10<sup>6</sup> PFU/ml, which was almost ten times larger than the value for E. coli K-12 (W3110) F<sup>+</sup> and E. coli C phages (Fig. 2). When HfrH F<sup>+</sup> was used for the examination, the resulting plaque counts reflected both the number of somatic phages and the number of F-specific ones. Theoretically, a count of F-specific phages could be obtained by subtracting the results for an F<sup>−</sup> strain from those for an isogenic F<sup>+</sup> strain. When the RNase was premixed with the plaque assay mixture, the E. coli HfrH (IAM 12017) F<sup>+</sup>-specific phage concentration was reduced from 2/3 to 1/2.
the plant except those infectious to the aerobic tank. No phages were detected in the effluent from the aerobic treatment using activated sludge. The phage concentration was higher in the returned sludge than that of the aerobic tank. The RNA phage was more stable than the DNA phage against predation by other microbes, protozoa or metazoa (9, 10). The increase in the attached phage concentration in the anaerobic tank was thought to be caused by the phage transition to solid particles from the liquid phase and the inflow of returned sludge from the secondary settling tank. The drastic reduction of the suspended phage in the aerobic tank would be due to adsorption onto the flock followed by predation by other microbes. The adsorbed phage settled down on the addition of PAC to the secondary settling tank and remained stable in the sludge. Almost no phage was detected in the supernatant of the secondary settling tank and effluent.

As far as we know, the seasonal change and fate of coliphage in a domestic wastewater treatment plant have not been well studied. The wastewater treatment process investigated in this study was typical of facilities in Japan with a daily capacity of 28,000 m$^3$ for 200,000 inhabitants. No remarkable seasonal change in the concentration of phage in the influent was observed during the ten-month test period. Even though the concentration in the influent was estimated at 1000–10,000 PFU ml$^{-1}$, almost no phage was detected in the effluent. Aerobic treatment using activated sludge and/or the addition of flocculants such as PAC was effective in the removal of coliphage, an index of enteric viral pollution.

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