Research for Pathogens in Water Environment and the Countermeasures in Sewerage

Seiichiro OKAMOTO*, Mamoru SUWA, Kensuke SAKURAI
Recycling Research Team, Material and Geotechnical Engineering Research Group, Public Works Research Institute (PWRI)

1. Introduction

In recent years, the frequent outbreak of communicable diseases caused by cryptosporidium, norovirus, or antibiotic resistant bacteria in Japan has become a public issue. In 1996, an outbreak of cryptosporidium occurred in Ogose town in Saitama Prefecture, infecting 9,000 people. And more recently, many infections caused by norovirus have occurred during the winter.

These pathogens discharged by infected people often flow into wastewater treatment plants (WWTPs) through sewer systems. Pathogens which cannot be removed by WWTPs are then discharged into public water bodies. Important ways to ensure the hygienic safety of public water bodies are to clarify the fate of pathogens in the water environment, wastewater, and treated wastewater and to develop effective measures to deal with them in sewage systems.

2. Study of cryptosporidium measures in sewage systems

In response to the cryptosporidiosis in Ogose town, it was recognized that cryptosporidium measures were necessary in sewage systems in order to conserve sources of water for public supply systems and to ensure the safety of water environments. And based on the results of a survey by the Public Works Research Institute (PWRI), the Study Committee on cryptosporidium in Sewage Systems has completed and released its final report (Japan Sewage Works Association, March 2000).

The final report summarized the following points.

- State of cryptosporidium concentration in wastewater and treated wastewater
- Evaluation of cryptosporidium removal effectiveness of the wastewater and sludge treatment processes
- Methods of measures for cryptosporidium in sewage systems
  - Ensuring safety of water bodies receiving final effluent of treated wastewater

* 1-6, Minamihara, Tsukuba, Ibaraki, 305-8516 JAPAN
  e-mail: s-okamoto@pwri.go.jp
Ensuring safety of sewage system employees

3. Profiles of norovirus in wastewater, treated wastewater, and river water

A practical method of detecting norovirus did not exist until recently, so its actual state was not adequately understood. In recent years, a detection method based on the polymerase chain reaction (PCR) method has come into wide use, and various bodies have applied it to conduct surveys.

The PWRI has used data concerning wastewater flowing into WWTPs, treated wastewater in public water bodies, and small rivers in regions with varying provision of sewerage treatment systems to study the relationship of norovirus (G1, G2) with chemical markers during the epidemic season.

The norovirus concentration detected differed greatly during the non-epidemic period and epidemic period, with the level of contaminants lowered during the non-epidemic period in both the wastewater and river water. And a specific correlation was found between norovirus and caffeine or total sterol. For this reason, measurement of the concentration of chemical markers has suggested that it is possible to estimate, to a certain degree, the state of maximum contamination during the norovirus epidemic period.

4. Characteristics of removal of norovirus from WWTPs

The PWRI has, in order to clarify the norovirus removal effectiveness of the activated sludge treatment method or the sand filtration method and to improve the removal rate of wastewater treatment processes, used a pilot plant to perform continuous removal tests by the activated sludge treatment method and sand filtration method to evaluate the removal rate of each method. The results are shown below.

- From December to March, which is the epidemic season, the norovirus concentration in influent wastewater ranges from $10^6$ to $10^7$ copies/L level for both G1 and G2 types.
- At the pilot plant, the activated sludge treatment method removal rates were, 0.6 to 1.6log for removal of G1 and 1.1 to 2.1log for G2, while the sand filtration method removal rate was about 0.6log for G2.
- The removal rates achieved by the activated sludge method with chemical addition, (adding poly-aluminum-chloride (PAC), 5mg-AL/L) were 0.6 to 2.6log (G1) and 1.4 to 2.9log (G2), and by 10mg-AL/L, 2.1 to 3.2log and 2.5 to 3.5log respectively. It was clarified that treating the addition concentration of PAC as 10mg-AL/L raised the removal rate to a maximum of between 2.1 and 2.5log.
The removal rate of G2 by sand filtration with chemical addition method (3mg-AL/L) is from 1.9 to 2.4log and higher: a maximum removal rate improvement of 1.8log over the sand filtration method.

It has been reported that the results of a survey at a trial facility by the Japan Sewage Works Agency show that norovirus has almost never been detected in water which has been treated by the membrane bioreactor (MBR).

5. Standards concerning viruses in treated wastewater

In Japan, there are no final effluent standards for viruses including norovirus in sewage systems. The major reasons for this are that the presence/absence of pollutants are not quantitatively clarified by present detection methods and that the state of viruses in both wastewater and in treated wastewater is not fully understood.

6. Future prospects

In the future, it will be necessary to continue to clarify the state of pathogens in environmental water and in treated wastewater and survey research on the removal of pathogens in treated wastewater. And it will also be necessary to research quantitative evaluations of the safety of viruses using the risk evaluation method.

The Ministry of Land, Infrastructure, Transport and Tourism (MLIT) established the Study Committee on norovirus in Sewage Systems in 2008 to perform studies focused on the preparation of guidelines concerning norovirus removal technologies etc.
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Seiichiro Okamoto
Recycling Research Team
Public Works Research Institute

Outline

- Background
- Study of *cryptosporidium* measures in sewage systems
- State of *norovirus* (NV) in wastewater, treated wastewater and river water
- Characteristics of removal of NV from wastewater treatment plants (WWTPs)
State of infectious diseases caused by pathogens in Japan

In Japan, *cryptosporidium*, *norovirus*, and other antibiotic resistant bacteria cause frequent infectious diseases

- *cryptosporidium* outbreak in Ogose town (JPN) (1996)
  - More than 9,000 people were infected
- Spread of *norovirus* (NV) nationwide in recent years
  - First in terms of food poisoning cases (2006)
  - Second in number of cases (2006)

Cryptosporidiosis outbreak in Ogose town (1996)

A large-scale outbreak caused 9,000 people to suffer from diarrhea and stomach pain.

- *Cryptosporidium* gets mixed with the tap water from the contaminated river water.
- There was a wastewater treatment facility upstream from water intake.
- *Cryptosporidium* propagated in the water cycle (wastewater -- Intake).
Study of *cryptosporidium* measures for sewage systems

- In response to Ogose outbreak, *cryptosporidium* for sewage systems were also studied.
- The Study Committee on cryptosporidium in Sewage Systems completed and announced its final report (2000)

Outline of the final report

- State of *cryptosporidium* concentration in wastewater and treated wastewater
- Evaluation of *cryptosporidium* removal effectiveness of the wastewater and sludge treatment processes
- Methods of measures for *cryptosporidium* in sewage systems
  - Ensuring safety of water bodies receiving effluent of treated wastewater (normal times and during an outbreak)
  - Ensuring safety of sewage system employees

**Adding flocculant (10mg/L)**

1/1000 of the concentration (activated sludge treatment)
State of *norovirus* (NV) in river water, wastewater and treated wastewater

*In Japan, food poisoning caused by NV is the leading cause: 27,616 patients out of total of 39,026 patients (71.0%).*

*Of a total of 1,491 outbreaks, 449 outbreaks (33.5%), second to *campylobacter jejuni/* campylobacter coli* (645 outbreaks)*

Occurrence during the past 6 years.

<table>
<thead>
<tr>
<th>Year</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
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</thead>
<tbody>
<tr>
<td>Total outbreaks</td>
<td>269</td>
<td>268</td>
<td>278</td>
<td>277</td>
<td>274</td>
<td>499</td>
</tr>
<tr>
<td>Total patients</td>
<td>7,358</td>
<td>7,961</td>
<td>10,603</td>
<td>12,537</td>
<td>8,727</td>
<td>27,616</td>
</tr>
<tr>
<td>Fatalities</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(Ministry of Health, Labor, and Welfare Web Site: quoted from norovirus Questions and Answers)
Characteristics of NV

- An extremely small round structured virus with diameter of about 38nm, discovered in victims of a 1968 gastroenteritis epidemic.
- Heating at 60°C for 30 minutes does not destroy its infectiousness.
- Its genotypes are classified as G1 and G2, which are communicated to people.
- It cannot be detected by the cell culture method. Difficulty to judge the activity.

Characteristics of NV (cont’d)

- Symptoms continue for only 1 to 3 days, but include violent vomiting, and later diarrhea and fever etc.
- It is known to be a virus which is a major cause of food poisoning and gastroenteritis through raw oysters (now rare) in the winter.
- In recent years, a high percentage of cases have been caused by person to person infection.
NV measurement method: Why the real time PCR method?

- cell culture method
  - impossible to measure the infectious capacity of NV
- electronic microscope, antigen-antibody reaction
  - sensitivity is low and quantitative evaluation is impossible.

- methods of rapidly and easily detecting and measuring it with high sensitivity should be developed.

Real time PCR equipment

Real time PCR ----
Technology which amplifies, monitors in real time, and analyzes (quantifies) a specified gene

(Benefits)
(1) Can accurately quantify a specified gene.
(2) Can perform analysis quickly and easily.

Example of a real time PCR System

- Applied Biosystems 7500
- LightCycler
- Smart Cycler
Procedure for quantification

- negatively charged membrane
- polyethylene glycol (PEG)

extract RNA, refine

convert the RNA to DNA

reverse transcriptase reaction

amplifies and quantifies a specified gene

real time PCR (quantification)

State of NV in river water

- Example of a survey by the PWRI -

- The coverage ratio of sewage systems varied between the rivers
  - At NT2, the coverage ratio of sewage systems is almost 100 %, and the river water is mainly spring water.
  - At OT2 and OT5, the coverage ratio is low and domestic wastewater is treated in septic tanks then discharged into the river
- Every six hours, water was sampled from 3 small rivers then analyzed
State of NV in river water
- Example of a survey by the PWRI -

- During the non-epidemic period (July), both G1 and G2 were below the quantification limits (3.8 x 10^3 copies/L).
- During the epidemic period (Jan.) both G1 and G2 were detected at concentrations of 10^5 copies/L at OT2.
- The detected concentration varies greatly between the epidemic and non-epidemic periods.

### Relationship of NV with chemical markers

- A correlation of NV with **caffeine** and **total sterol**
  - Correlation coefficients for G1 are 0.86 and 0.73 for caffeine and total sterol respectively, and for G2, 0.80 and 0.67 for caffeine and total sterol respectively.
- The NV concentration can be estimated based on the concentrations of the chemical markers: caffeine and total sterol.

During the epidemic period
NV Removal Tests at the Pilot Plant

- Continuous removal test of the activated sludge treatment method
  - A pilot plant consisting of an aeration tank (capacity 100 L) and primary and secondary sedimentation tanks (capacities 50 L) etc. was used for the tests.

Schematic of the Pilot Plant

Influent wastewater was supplied continuously

- Chemical added (poly-aluminum chloride (PAC)), at a rate of 5 and 10 mg-AL/L (System 1)
- Sampling point
- MLSS 2,000 mg/L
- HRT 8 hours
- Activated sludge method with chemical addition (System 1)
- Standard activated sludge method (System 2)

Secondary effluent

- Sand filtration with chemical addition (Run 2)
- PAC added at a rate of 3 mg-AL/L (Run 2)

Sampling point

Sedimentation tanks

Chemical added (poly-aluminum chloride (PAC)), at a rate of 5 and 10 mg-AL/L (System 1)
Results of testing

- Changes of NV concentration (measures of NV started in late December)
- Quantity of PAC added increased from 5 mg/L to 10 mg/L (System I)

Results of testing (removal rates)

- Standard activated sludge treatment
  ---- removal rate from 0.6 log to 2.1 log
- Chemical addition (10mg-Al/L)
  ---- removal rate rose to about 3 log
Improving NV removal effectiveness by sand filtration

- Sand filtration at filtration rate of 200 m/day achieved G2 removal of 0.6 log (74%)
- Sand filtration with chemical addition (3 mg-AL/L) achieved removal rate from 1.4 to 2.4 log or higher
- Addition of PAC achieved removal rate from 1.4 log to 2.4 log.

<table>
<thead>
<tr>
<th>removal rates (log)</th>
<th>Average removal rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV (G1)</td>
<td>NV (G2)</td>
</tr>
<tr>
<td>Sand filtration (Run 1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sand filtration with chemical addition (Run 2)</td>
<td>1.4 - 2.7</td>
</tr>
</tbody>
</table>

Conclusion
(Removal tests at the Pilot Plant)

- The NV concentration in influent wastewater was $10^6$ to $10^7$ copies/L

- The NV removal rates
  - Activated sludge method ---- 0.6 log to 2.1 log.
  - Activated sludge with chemical addition
    ---- 0.6 log to 2.9 log (5 mg-AL/L)
    ---- 2.1 log to 3.5 log (10 mg-AL/L)
  - Sand filtration ---- about 0.6 log (G2)
  - Sand filtration with chemical addition (3 mg-AL/L)
    ---- 1.4 log to 2.4 log
Future challenges

- In Japan there are no final effluent standards for viruses in sewage systems.
  - Present detection methods cannot quantitatively detect the presence or absence of pollutants.
  - The state of viruses in wastewater and in treated wastewater are not fully understood.

- The MLIT* established the Study Committee on viruses in Sewage Systems (2008)
  - To perform studies focused on the preparation of guidelines concerning NV removal technologies etc.

*MLIT: Ministry of Land, Infrastructure, Transport and Tourism

Future survey challenges

- Clarifying the state of pathogens and conduct surveys of the removal of pathogens in wastewater treatment plants.
- Evaluations of the safety of viruses using the risk evaluation method.

Disinfection diactivates NV?

Combined Sewer Overflows (CSOs) concentration of NV?
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Thank you for your kind attention.