Membrane separation of indigenous noroviruses from sewage sludge and treated wastewater

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Abstract In this study, feasibility of membrane separation for the removal of indigenous noroviruses (NVs) is evaluated. The indigenous NV gene was never detected from ultrafiltration (UF) permeates of sewage sludge and treated wastewater. Indigenous NV gene was also not detected from permeates of sewage sludge and treated wastewater by microfiltration (MF) with a pore size of 0.1 μm (MF0.1). Even though the pore size of MF (0.1 μm) was much larger than the diameter of virus particle (approximately 30–40 nm), more than 4-$\log_{10}$ reduction value (LRV) at maximum was achieved by membrane separation with MF0.1. NV genes were often detected from permeates of sewage sludge and treated wastewater by MF with a pore size of 0.45 μm (MF0.45), although the maximum $\log_{10}$ reduction values were more than 3.59 for sewage sludge and more than 2.90 for treated wastewater. It is important to verify factors determining the removal efficiency of viruses with MF membranes.

Keywords F-specific RNA bacteriophage; $\log_{10}$ reduction value; microfiltration; noroviruses; polioviruses; realtime RT-PCR; ultrafiltration

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Introduction

Noroviruses (NVs) are known to be the leading etiological agents of acute gastroenteritis over the world. NVs are excreted from patients of infectious gastroenteritis, and flow into wastewater treatment plants. It is crucial to efficiently remove NVs in wastewater treatment, or the water environment becomes severely contaminated. Proliferation of NVs in the water environment has often been reported (Haramoto *et al.*, 2005; Lodder and Husman, 2005; Ueki *et al.*, accepted). Many researchers indicated that conventional water and wastewater treatments have generally shown poor performances in virus removal and inactivation (Leong, 1983; Gerba, 1984; Keswick *et al.*, 1985), and that would be one of the main reasons why contamination of the water environment with pathogenic viruses has often been reported. A new scheme for removing viruses from domestic wastewater will need to be introduced to prevent the water environment from further contamination with NVs.

In recent years, membrane separation has received much attention as an effective procedure for removing contaminants from water. In particular, virus removal is achieved with membrane filtration and has advantages in water and wastewater treatments because virus particles can be physically eliminated. The efficacy of the membrane separation for virus removal has been reported with regard to pathogenic viruses including Hepatitis A virus (Vaidya *et al.*, 2004) and polioviruses (Madaeni *et al.*, 1995), but the application of the membrane separation for the removal of indigenous NVs has not been evaluated.

In this study, the feasibility of the membrane separation in the removal of indigenous NVs from sewage sludge and treated wastewater was evaluated. Sewage sludge and

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treated wastewater were sampled from a domestic wastewater treatment plant, and indigenous NVs were removed with microfiltration (MF) or ultrafiltration (UF) membranes. Concentrations of NVs in source samples and permeates were determined with quantitative-PCR for the NV partial gene, and the removal efficiency of indigenous NVs was compared with that of indigenous F-specific RNA bacteriophages and inoculated poliovirus type 1 (PV1).

Materials and methods

Membrane filtration of sewage sludge and treated wastewater

Eleven samples of sewage sludge and treated wastewater were taken between February 2003 and June 2004 from a municipal wastewater treatment plant in Matsushima city, Miyagi prefecture, Japan. This wastewater treatment plant employs the oxidation ditch system for the treatment of approximately 4,400 m^3 per day of domestic wastewater. The average and standard deviation of suspended solids were 2,550 and 610 mg/L ($n = 11$) for sewage sludge samples and 3.37 and 1.85 mg/L ($n = 11$) for treated wastewater samples, respectively. Sewage sludge and treated wastewater were filtered with mixed cellulose ester MF membranes with a pore size of 0.45 μm (MF0.45) (A045A090C, Toyo Roshi Kaisha Ltd., Tokyo, Japan), pore size of 0.1 μm (MF0.1) (A010A090C, Toyo Roshi Kaisha Ltd., Tokyo, Japan) and regenerated cellulose UF membranes with the molecular weight cut off (MWCO) of 100,000 (YM100, Millipore, Billerica, Mass., USA). Membranes were set in a filter holder (03-5442-9719, Life Science), and source samples were introduced into the filter holder at a constant pressure with nitrogen gas (Figure 1). The pressure values were 50 kPa for MF membranes and 100 kPa for UF membrane. In the PV1 inoculation test, laboratory-cultured PV1 was added to samples at the concentration of 10^4 to 10^5 PFU/mL prior to the filtration. New membrane was used for each trial.

Quantification of NV gene, F-specific RNA bacteriophages and PV1

Viruses in sludge samples were recovered with an enzymatic virus elution (EVE) method as previously described (Sano *et al.*, 2003). In the EVE method, hydrolytic enzymes and cation exchange resin (CER) were used for the enhancement of virus elution from solids. Since samples prepared with the EVE method have no inhibitory effects on cell culture (Sano *et al.*, 2001) and RT-PCR (Sano *et al.*, 2003), the EVE method is a powerful tool for the detection of viruses adsorbing to solids. Viruses in treated wastewater and permeates were concentrated with polyethylene glycol precipitation according to Lewis and Metcalf (1989). Viral RNA extraction and complementary DNA synthesis were

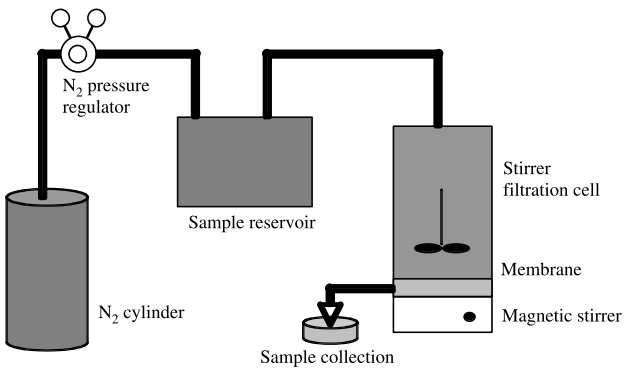

Figure 1 Stirred filtration cell set-up

performed as previously described (Ueki *et al.*, 2005). Complementary DNA of the NV capsid gene (GI and GII genogroups) was quantified with real-time reverse transcription (RT)-PCR (Kageyama *et al.*, 2003). The sum of gene number of NV GI and GII was described as the number of NV genes in this study. The indigenous F-specific RNA bacteriophage was assayed using *Salmonella typhimurium* WG49 as a host strain (International Organization for Standardization, 2000). Infectious PV1 was quantified with the plaque method using BGM cells as previously described (Sano *et al.*, 2001).

Efficiency of virus reduction with membrane separation

The efficiency of virus reduction with membrane separation was expressed by the $\log_{10}$ reduction value (LRV), which is defined as:

$$\mathrm{LRV} = -\log_{10} Cp/Cs \quad (1)$$

where Cs is the quantity of NV gene, F-specific RNA bacteriophage or infectious PV1 in the source sample and Cp is the quantity of NV gene, F-specific RNA bacteriophage or infectious PV1 in permeate.

Results and discussion

Membrane separation of viruses from sewage sludge

Table 1 shows LRVs for membrane separation of the indigenous NV gene, F-specific RNA bacteriophage and inoculated PV1 from sewage sludge, respectively. The indigenous NV gene, F-specific RNA bacteriophage and inoculated PV1 were never detected from UF permeates of sewage sludge. The MWCO of the UF is 100,000, which is approximately 100 times smaller than the molecular weight of virus particles (approximately 10 million).

These results mean that the UF membrane could physically eliminate NVs and polioviruses from sewage sludge, and more than 4 or 5 LRV at a maximum could be expected, respectively. This elimination of virus particles by UF membrane has significance because other treatment barriers may not be capable of similar removal or inactivation of pathogenic viruses in wastewater.

The indigenous NV genes and F-specific RNA bacteriophage were not detected from MF0.1 permeates of sewage sludge (Table 1). Even though the pore size of MF0.1 was much larger than the diameter of viruses (approximately 30 to 40 nm), a high efficiency of virus reduction (more than 4 LRV at maximum) was achieved by membrane separation with MF0.1. Part of the reason why indigenous viruses are well removed could be that these viruses are embedded in solids, and physically excluded by the membrane surface. On the other hand, inoculated PV1 was detected from all MF0.1 permeates, with the average of the LRV of 4.0. Varying concentrations of naturally occurring viruses in the influent limited detection limits in the permeate. We considered that the adsorption of viruses to sludge solids has a crucial rule in removing viruses with microfiltration.

The indigenous NV gene was detected six times in eleven trials from MF0.45 permeates of sewage sludge, although the maximum LRV was more than 3.59 (Table 1). The indigenous F-specific bacteriophage was detected three times in eleven trials from MF0.45 permeates of sewage sludge, and the maximum LRV was more than 4.89. These results indicate that the removal efficiency of viruses with MF0.45 fluctuates (e.g. in Table 1, the LRV of NVs was 0.47 to 2.23) and might depend on several factors, such as solid quantity and composition. MF0.45 has been preferable for application in water and wastewater treatment because of practical reasons, so it is important to verify factors determining the removal efficiency of NVs.

Table 1 Log_{10} reduction values (LRV) of naturally occurring viruses in sewage sludge with membrane separation

	NVs										
Trial	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
UF[a]	>4.44[b]	>3.00	>2.91	>1.93	>3.01	>2.48	>3.56	>3.59	>2.17	>1.90	>1.04
MF0.1[c]	>4.44	>3.00	>2.91	>1.93	>3.01	>2.48	>3.56	>3.59	>2.17	>1.90	>1.04
MF0.45[d]	2.23[e]	2.07	2.10	>1.93	0.47	>2.48	1.56	>3.59	>2.17	>1.90	0.44
F-specific bacteriophage											
UF	>4.89	>3.23	>4.11	>4.45	>3.64	>4.68	>4.53	>4.20	>4.01	>4.85	>4.28
MF0.1	>4.89	>3.23	>4.11	>4.45	>3.64	>4.68	>4.53	>4.20	>4.01	>4.85	>4.28
MF0.45	>4.89	3.70	2.59	>4.45	>3.64	>4.68	4.53	>4.20	>4.01	>4.85	>4.28
Poliovirus type 1 (spiked)											
					Average (SD)						
UF	>4.90	>5.10	>5.10	>3.30							
MF0.1	4.00	5.20	4.70	2.70	4.00 (0.1)						
MF0.45	2.00	3.20	3.50	0.90	2.4 (0.2)						

[a]Ultrafiltration membrane molecular weight cut-off 100,000; [b]values with inequality sign calculated by dividing the detection limit value by quantity of virus/detected genes in sewage sludge; [c]microfiltration membrane pore size 0.1 μm; [d]microfiltration membrane pore size 0.45 μm; [e]values without inequality sign calculated by dividing quantity of virus/detected genes in permeate by virus quantity in sewage sludge

Table 2 Log_{10} reduction values (LRV) of naturally occurring viruses in treated wastewater with membrane separation

	NVs						
Trial	**1**	**2**	**3**	**4**	**5**	**6**	**7**
UF[a]	>3.91[b]	>2.20	>2.86	>3.31	>3.37	>1.76	>2.90
MF0.1[c]	3.28[d]	>2.20	>2.86	>3.31	>3.37	>1.76	>2.90
MF0.45[e]	0.82	0.04	>2.86	1.32	2.26	−0.83	>2.90
F-specific bacteriophage							
UF	>2.11	>2.14	>1.62	>1.71	>0.80	>2.16	
MF0.1	>2.11	>2.14	>1.62	>1.71	>0.80	>2.16	
MF0.45	0.93	0.36	1.62	>1.71	>0.80	0.93	
Poliovirus type 1 (spiked)							
					Average (SD)		
UF	>4.90	>4.70	>4.70	>3.30			
MF0.1	2.10	1.80	2.10	2.10	2.00 (0.1)		
MF0.45	2.20	1.60	1.60	0.40	1.5 (0.7)		

[a]Ultrafiltration membrane molecular weight cut-off 100,000; [b]values with inequality sign calculated by dividing the detection limit value by quantity of virus/detected genes in treated wastewater; [c]microfiltration membrane pore size 0.1 μm; [d]microfiltration membrane pore size 0.45 μm; [e]values without inequality sign calculated by dividing quantity of virus/detected genes in permeate by virus quantity in treated wastewater

Membrane separation of viruses from treated wastewater

Table 2 shows LRVs for membrane separation of the indigenous NV gene, F-specific RNA bacteriophage and inoculated PV1 from treated wastewater. These viruses and phages were never detected from UF permeates of treated wastewater as well as the UF permeates of sewage sludge. Since the UF membrane could physically trap virus particles, it would be possible to eliminate viruses in samples with small amount of solids compared with sewage sludge.

The indigenous NV genes were detected from MF0.1 permeates of treated wastewater once, while F-specific RNA bacteriophage were not detected from MF0.1 permeates of treated wastewater, as well as the MF0.1 permeates of sewage sludge. Since its pore size is larger than diameter of virus particles (approximately 30 to 40 nm), the adsorption of viruses to suspended solids is important to remove viruses with microfiltration. It is considered that indigenous viruses are adsorbed to suspended solids and physically excluded by clogging these solids on the membrane surface, even though the average value of suspended solids in treated wastewater (3.37 mg/L) was approximately one hundredth of those in sewage sludge (2,550 mg/L). On the other hand, inoculated PV1 was detected from all MF0.1 permeates with the average of the LRV of 2.0. The short contact time of the inoculated PV1 may have affected the virus removal with MF0.45. This result also supports the fact that the adsorption of viruses to suspended solids is important to remove these viruses with microfiltration.

The indigenous NV gene was detected five times in seven trials from MF0.45 permeates of treated wastewater, and the maximum LRV was more than 2.90 (Table 2). The indigenous F-specific bacteriophage was detected four times in six trials from MF0.45 permeates of treated wastewater, and the maximum LRV was more than 2.14. The removal of indigenous viruses from treated wastewater with MF0.45 is more difficult than that from sewage sludge, which would be attributed to the small amount of suspended solids in treated wastewater compared with those in sewage sludge. The coagulation process prior to the membrane filtration would improve the virus removal with microfiltration, in which viruses are involved in flocs during the coagulation process and removed by trapping flocs with microfiltration. The same thing can be said for membrane

filtration of sewage sludge, and membrane separation activated sludge process combined with coagulation process could show good performance in the virus removal.

Conclusions

The indigenous NV gene, the indigenous F-specific RNA bacteriophage and inoculated PV1 were not detected from UF permeates of sewage sludge and treated wastewater, which means that the UF membrane could physically eliminate viruses from sewage sludge and treated wastewater. The indigenous NV gene and F-specific RNA bacteriophage were not detected from MF0.1 permeates of sewage sludge and treated wastewater. Even though the pore size of MF0.1 was much larger than the diameter of virus particles (approximately 30 to 40 nm), high efficiency of virus reduction (more than 4 LRV at maximum) was achieved by membrane separation with MF0.1. The adsorption of viruses to suspended solids has a crucial rule in the virus removal with microfiltration. The indigenous NV gene was often detected from MF0.45 permeates of sewage sludge and treated wastewater, although the maximum LRVs were more than 3.59 for sewage sludge and more than 2.54 for treated wastewater. These results indicate that the removal efficiency of NVs fluctuates and might depend on several factors. It is important to verify factors determining the removal efficiency of NVs.

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