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ORIGINAL PAPER

How Fiber Breakage Reduces Microorganism Removal in Ultrafiltration for Wastewater Reclamation

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Abstract

Ultrafiltration (UF) membranes are increasingly being used for wastewater reclamation treatment for their high removal of pathogens and suspended solids. However, breakage of UF membrane fibers could allow leakage of pathogens into the permeate and create health risks in the use of reclaimed water. Here, we assessed the $\log_{10}$ reduction value (LRV) of human enteric viruses and microbial indicators of new and aged UF modules in a pilot-scale UF process to evaluate the influence of fiber breakage. Norovirus genotypes I and II, Aichi virus, and *Escherichia coli* were not detected in any permeate samples of intact UF modules, but were detected in samples of damaged UF modules. LRVs of all microorganisms assayed decreased as fiber breakage of new UF modules increased, with maximum decreases of > 3.3 $\log_{10}$. Fiber breakage in the aged UF modules did not decrease LRVs of somatic coliphages and MS2, but breakage in the new UF modules did decrease them. Intact new UF modules gave higher LRVs than intact aged UF modules. When the LRV of intact UF module was assumed to be 1 or 2 $\log_{10}$, increasing fiber breakage did not significantly decrease the predicted LRV, but when it was ≥ 3 $\log_{10}$, it did decrease LRV, in good agreement with measured LRVs in the degraded UF modules. These results suggest that the LRV of intact UF modules affects the decrease in LRV and confirm the leakage of human enteric viruses following fiber breakage in UF modules of different ages in the UF process of wastewater reclamation.

Keywords Ultrafiltration · Integrity · Microorganism removal · Fiber breakage · Wastewater reclamation

Introduction

Increasing global water shortages have increased the need for wastewater reclamation. Pathogenic microorganisms present in domestic wastewater must be removed for safe reuse (Sano et al. 2016; Gerba et al. 2018). Ultrafiltration (UF) membranes are increasingly being used for wastewater reclamation treatment (Reeve et al. 2016; Lee et al. 2017a, b, 2018) for their high levels of removal of pathogens and suspended solids. In particular, coliform bacteria and protozoan cysts (*Giardia* and *Cryptosporidium*) are in general highly retained by UF (Jacangelo et al. 1995, 1997; Ferrer et al. 2015). Norovirus (NoV), a human enteric virus, is also removed to non-detectable levels (Qiu et al. 2015; Lee et al. 2017a). Thus, UF can reduce the dose of disinfectant needed.

However, if UF membrane integrity is compromised, pathogens could pass through and enter the reclaimed water supply. Several reasons, including chemical corrosion, faulty installation and maintenance, membrane stress and strain, and damage by sharp objects not removed by pretreatment, can cause fibers to break or degrade (Guo et al. 2010; Antony et al. 2012). Fiber failures occur at an annual rate of 1 out of 10,000–1,000,000 fibers (Gijsbertsen-Abrahamse et al. 2006). These rates correspond to at least 1 broken fiber in every full-scale wastewater reclamation plant a year, as most plants install over 100 UF modules. Failures could therefore increase health risk posed by the use of reclaimed water. However, only three studies have investigated the impact of membrane damage on the efficiency of pathogen removal in

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laboratory-scale UF modules, a bench-scale UF plant, and a pilot-scale membrane bioreactor using microbial indicators such as total coliform bacteria and the MS2 bacteriophage (Brehant et al. 2010; Antony et al. 2014; Hirani et al. 2014), whereas to date no study has evaluated the influence of damage in a pilot-scale UF plant on the passage of indigenous human enteric viruses or of microbial indicators. Such investigation is necessary.

Membrane fibers can break at various ages of an installation. Weak fibers in a new membrane can break during early use. Fibers in aged membranes can break because of accumulated chemical degradation, oxidative chemical cleaners, or air sparging and backwashing. The effects of fiber breakages on the efficiencies of pathogen removal can differ between new and aged membranes owing to differences in pathogen removal performance between them. Previous studies showed that the virus removal efficiencies of new membranes (< 1 year) were higher than those of aged membranes (> 2–6 years) in pilot- and full-scale UF plants (Reeve et al. 2016; Lee et al. 2017a, 2018). Several reasons, such as virus adsorption to new membrane surfaces, irreversible fouling of aged membranes, and pore expansion of aged membranes, can alter virus removal efficiencies (Van Voorthuizen et al. 2001; Antony et al. 2014; Reeve et al. 2016). Therefore, it is necessary to compare the influence of fiber breakage between new and aged UF modules.

Here, we investigated the influence of fiber breakage in new and aged UF modules on the removal of a bacterial indicator (*Escherichia coli*), human enteric viruses (NoV genotypes I and II: GI and GII), potential viral indicators (pepper mild mottle virus: PMMoV; Aichi virus: AiV), and a somatic coliphage (SOMCPH) in pilot-scale UF plants. Indigenous PMMoV and AiV have been suggested as potential viral indicators for the evaluation of virus removal efficiency because they are highly prevalent and show no seasonal change of concentration in feed water (Kitajima et al. 2014; Asami et al. 2016; Schmitz et al. 2016; Lee et al. 2017a). We also spiked samples with MS2 to see the effect on viral detection by plaque assay and real-time reverse-transcription polymerase chain reaction (RT-qPCR). Furthermore, we formulated an equation to predict the removal efficiency following fiber breakage so as to investigate the effects of different factors on the decrease in removal efficiency.

Materials and Methods

UF Fiber Breakage Tests

We investigated the effect of fiber breakage on the removal of microorganisms in two pilot-scale wastewater reclamation plants of 10 m^3/day capacity (WWTP A, Shiga Prefecture; WWTP B, Okinawa Prefecture), which use conventional activated sludge treatment. The secondary effluent was used as feed water. The characteristics and operational parameters of the UF pilot plants are shown in Table 1.

Table 1 Characteristics and operational parameters of the UF pilot plants

Nominal pore size	0.01 μm
Molecular weight cutoff	150 kDa
Total surface area	11.5 m^2
Membrane materials	Polyvinylidene fluoride
No. of fibers/module	1000
Operation mode	Dead-end (stable flux operation)
Flux	2 m/day
Backwash	Water and air for 1 min each every 30 min
Chemical cleaning	300 mg Cl_2/L (20 min soak) once a day
Maximum operating pressure	200 kPa

Table 2 Specifications of the UF modules used in the fiber breakage tests

		Period of use	Transmembrane pressure (kPa)	Site
New UF	UF 1	2 weeks	27–38	WWTP A
	UF 2	1 week	22–38	WWTP A
Aged UF	UF 3	> 4 years	37–73	WWTP B
	UF 4	> 4 years	32–73	WWTP B

We used four UF modules of different periods of use (Table 2). New UF modules (UF 1 and 2) at WWTP A were less than 2 weeks old before the experiments. Aged UF modules (UF 3 and 4) at WWTP B were over 4 years old (Table 2). All the UF modules were from the same manufacturer and had undergone manufacturing process (HFU-2008; Toray, Shiga, Japan), except for WWTP. To simulate fiber breakage, we cut a fiber at the feed side near the permeate side, where breakage may have the most influence, by pulling it with a wire hook. Breakages of 0, 1, 3, 5, and 10 fibers per 1000 (0.0%, 0.1%, 0.3%, 0.5%, and 1.0% damage) were sequentially made to each module (Fig. 1). Following each breakage, feed and permeate samples were collected 15 min after UF was started (Fig. 1). MS2 was spiked into the feed water tank at 10^6–10^7 PFU/mL during these tests. The fiber breakage experiments were performed in the order of 0, 1, 3, 5, and 10 fibers and were subjected to backwash UF for each condition. Permeate samples were collected at the pipe near the permeate side, not from the permeate water tank. Thus, there was minimal to no risk of cross-contamination of pathogens and indicators between each condition.

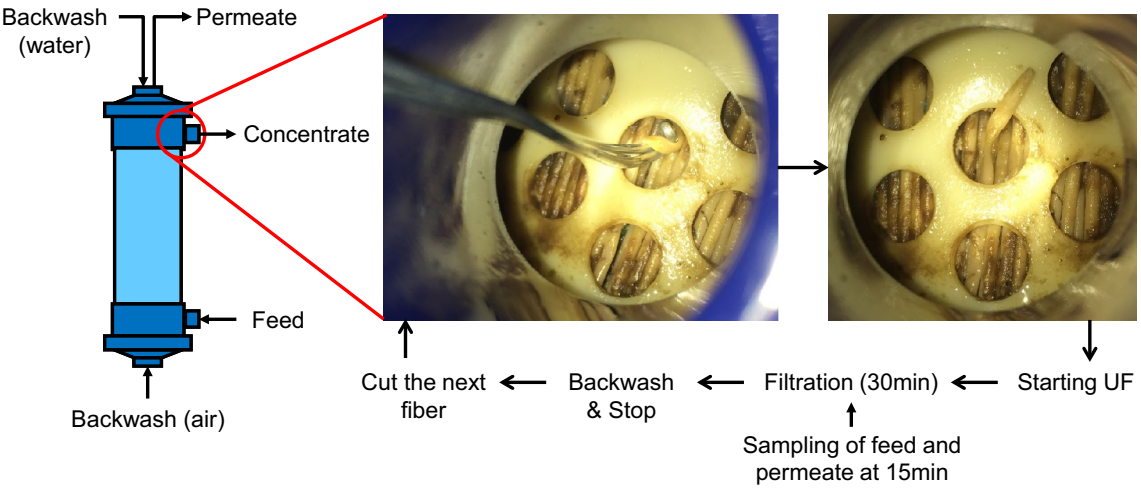

Fig. 1 Procedure for testing the effects of fiber breakage on LRV

The feed and permeate samples for measurement by RT-qPCR were concentrated immediately after sampling, using our previously described adsorption-elution method on a negatively charged membrane, to obtain 0.7 mL of virus concentrate from 1000 mL of sample (Lee et al. 2017a). Briefly, 2500 mM of magnesium chloride was added to the collected water samples to obtain a final concentration of 25 mM, and the samples were then passed through an electronegative membrane filter (90 mm diameter, 0.45 mm pore size; Millipore). Then, 200 mL of 0.5 mM sulfuric acid (pH 3.0) was passed through the membrane. Finally, 10 mL of 1.0 mM sodium hydroxide (pH 10.8) was passed through the membrane to elute the viruses. The eluate was recovered in a tube containing 50 µL of 100 mM sulfuric acid (pH 1.0) and 100 µL of 100 × Tris–EDTA buffer (pH 8.0) for neutralization. The eluate was further concentrated using a Centriprep YM-50 (nominal molecular weight cutoff of 50 kDa; Millipore) to obtain a final volume of approximately 0.7 mL. Samples for measurement by culture-based methods were held at 4 °C in a cooler with ice packs and transported to our laboratory.

Quantification of Enteric Viruses and Indicators

Quantification of Enteric Viruses

Enteric viruses (NoV GI and GII), potential viral indicators (AiV and PMMoV), and spiked viral indicator (MS2) were assayed by RT-qPCR. Viral RNAs were extracted from 140 µL of the concentrated samples using a QIAamp Viral RNA Mini Kit (Qiagen) to obtain 100 µL of RNA extract according to the manufacturer's protocol. The number of genome copies ("GC") of the extracted viral RNAs were quantified by RT-qPCR as described (Lee et al. 2017a). The number of GCs was determined from previously prepared standard curves. Separate standard curves were created in triplicate, with every plate using plasmid DNA that contained the targeted virus gene sequences to be amplified, with tenfold serial dilution (10^5–10^0 GC per reaction). The plasmid DNA was extracted from *E. coli* using QIAprep Spin Miniprep Kit (Qiagen). The concentration of extracted plasmid DNA was measured using a BioSpec-nano spectrophotometer (Shimadzu) and was converted into the number of GC based on Avogadro's number. Only data collected from plates whose standard curve had r^2 values of >0.95 and qPCR efficiencies of between 90 and 110% were accepted. The qPCRs were performed in duplicate and were considered positive only when both tubes fluoresced with sufficient intensity (< 10% of variation was allowed between duplicates), and the average cycle threshold value was not greater than 40, as recommended by the guidelines described elsewhere (Bustin et al. 2009). Two PCR tubes per sample of negative controls for each process (i.e., RNA extraction, RT and qPCR) were included to detect any false-positive results due to cross–contamination, but no false-positive qPCR signals were observed (data not shown). We assumed the quantification limit to be an estimated 1 GC per reaction in this study. The quantification limits of the feed and permeate samples were 2.0 $\log_{10}$ copies/L. When no increase in fluorescence was observed within 40 cycles, the sample concentration was classified as 'below the detection limit'. We used murine norovirus (MNV, strain S7-PP3, kindly provided by Dr. Y. Tohya, Nihon University, Kanagawa, Japan) as a sample process control to determine the efficiency of viral RNA extraction–RT-qPCR. MNV was propagated in RAW 264.7 (ATCC TIB-71) cells (American Type Culture Collection, Manassas, VA, USA). Before viral RNA extraction, 5.0 µL of MNV stock was spiked into 140 µL of concentrated sample or of pure water (control, no inhibition). MNV-RNA was co-extracted with the viral

RNAs, and the yield was determined by RT-qPCR (Kitajima et al. 2010). The recovery efficiency was calculated as:

$$\text{Recovery efficiency } (\%) = \frac{C}{C_0} \times 100 \quad (1)$$

where C is the copy number of MNV-cDNA per qPCR tube and C_0 is the copy number in the control. Recovery efficiencies of < 100% indicate losses of viral RNA during extraction or the occurrence of RT-PCR inhibition. Recovery efficiencies were determined for each sample, but they were not used to adjust the concentration of indigenous and spiked viruses.

Quantification of E. coli and SOMCPH

Concentrations of *E. coli* were quantified on XM-G agar (Nissui Seiyaku, Tokyo, Japan) according to the manufacturer's protocol. Concentrations of the SOMCPH were measured by conventional plaque assays in *E. coli* WG5 host cells as described (Lee et al. 2018).

MS2 Preparation and Quantification

F-specific RNA bacteriophage MS2 (NBRC 102,619, National Biological Resource Center, Japan) was propagated for 22–24 h at 37 °C in *E. coli* strain NBRC 13,965 host cells. The MS2 stock solution was purified as described previously (Lee et al. 2017a, b). The concentration of MS2 in the purified solution was 10^9–10^{10} plaque forming units (PFU)/mL according to the results of plaque assay (Lee et al. 2017a, b).

Prediction of Removal Efficiency Following Fiber Breakage

We calculated the $\log_{10}$ reduction value (LRV) as:

$$LRV = -log_{10}\left(\frac{C_p}{C_f}\right) \quad (2)$$

where C_f and C_p are the concentrations in the feed and permeate water. If C_p was below the detection limit, the quantification limit was used.

To investigate the effect of LRV of intact UF module on LRV decrease by fiber breakage, we calculated the predicted LRV (LRV_p) following fiber breakage as:

$$LRV_p = -log_{10}\left(\left(\frac{N_t - N_b}{10^{LRV_i}} + 1\right)/N_t\right) \quad (3)$$

where N_t is the total number of fibers, N_b is the number of broken fibers, and LRV_i is the LRV of the intact filter. This equation is explained in detail in Supplementary Information.

Table 3 Recovery efficiencies of spiked MNV in each sample

Sample	Rate of fiber breakage (%)	*n*	Geometric mean ± SD
Feed		20	92% ± 17%
Permeate	0.0	4	98% ± 11%
	0.1	4	93% ± 17%
	0.3	4	101% ± 8%
	0.5	4	101% ± 8%
	1.0	4	103% ± 6%

Table 4 Concentrations of indigenous enteric viruses and indicators expressed in $\log_{10}$ units per L of feed water during fiber breakage tests using UF 1–4

	No. positive/no. tested (%)	Mean ± SD (min.–max.) ($\log_{10}$ unit/L)
Virus[a]		
NoV GI (GC)	20/20 (100%)	3.0 ± 0.5 (2.4–3.9)
NoV GII (GC)	20/20 (100%)	4.8 ± 0.3 (4.4–5.4)
Indicator[a,b]		
PMMoV (GC)	20/20 (100%)	7.1 ± 0.6 (6.0–8.0)
AiV (GC)	15/20 (75%)	2.6 ± 0.1 (2.5–2.7)
E. coli (CFU)	20/20 (100%)	5.2 ± 0.5 (4.5–6.0)
SOMCPH (PFU)	20/20 (100%)	5.0 ± 0.5 (4.5–5.9)

[a]Indigenous viruses were measured by RT-qPCR

[b]Indigenous *E. coli* and SOMCPH were measured by culture assays

Results

Viral RNA Extraction and RT-qPCR Efficiency

High efficiencies of MNV recovery (92–103%) from all samples (Table 3) indicate that there was minimal viral RNA loss or RT-qPCR inhibition.

Viruses and Indicators in Feed Water

NoV GI and GII were detected in all feed water samples (100%) at concentrations of 2.4–3.9 (GI) and 4.4–5.4 (GII) $\log_{10}$ GC/L (Table 4). PMMoV was detected in all feed water samples at 6.0–8.0 $\log_{10}$ GC/L (the most numerous among the indigenous viruses tested). AiV was detected in 75% of feed water samples at 2.5–2.7 $\log_{10}$ GC/L. *E. coli* and SOMCPH were detected in all feed water samples (100%) at 4.5–6.0 $\log_{10}$ CFU/L and 4.5–5.9 $\log_{10}$ PFU/L, respectively.

Virus and Indicator Reductions in Fiber Breakage Tests

NoV GI and GII were not detected in any permeate samples from intact (0% breakage) UF 1–4, whereas they were detected in permeate samples from 0.1 to 1.0% breakage UF 2–4 (Fig. 2 and Table S1). LRVs of NoV GI and GII decreased as a result of UF fiber breakages. PMMoV was detected in all the permeate samples from UF 1–4 (Fig. 2). Its LRVs for intact (0% breakage) UF 1–4 were higher with new UF modules (UF 1 and 2) than with aged UF modules (UF 3 and 4). LRVs of PMMoV decreased as breakage of UF 1–3 (but not UF 4) increased (Fig. 2 and Table S1). AiV was not detected in any of the permeate samples from intact UF 1–4 (Fig. 2), but was detected in permeate samples from UF 4 (0.3% breakage) and UF 2 (1.0% breakage: Fig. 2 and Table S1).

Escherichia coli was not detected in any permeate samples from intact UF 1–4 (Fig. 3 and Table S2), but was detected in all permeate samples from UF 1–4 (0.1–1.0% breakage). LRVs decreased as the breakage of UF 1–3 increased, but the decrease in UF 4 was not consistent. SOMCPH was detected in all permeate samples from UF 1–4 (Fig. 3 and Table S2). LRVs of intact (0% breakage) UF 1–4 were higher in new UF modules (UF 1 and 2) than in aged UF modules (UF 3 and 4). LRVs of UF 1 and 2 decreased as breakage increased. However, LRV of UF 3 did not decrease and that of UF 4 decreased only slightly. LRVs of spiked MS2 determined by culture assay (PFU) were similar to those of SOMCPH, also determined by culture assay (Fig. 3 and Table S2). LRVs of intact (0% breakage) UF 1–4 were higher in new UF modules (UF 1 and 2) than in aged UF modules (UF 3 and 4). LRVs of UF 1 and 2 decreased as breakage increased, whereas those of UF 3 and 4 did not, as seen with SOMCPH. In contrast, LRVs of spiked MS2 in intact UF 1 and 2 (new UF modules) were lower by RT-qPCR (GC) than by PFU (Fig. 3 and Table S2), but those in intact UF 3 and 4 (aged UF modules) by RT-qPCR were similar to those by PFU. Furthermore, the LRV decrease as breakage increased in

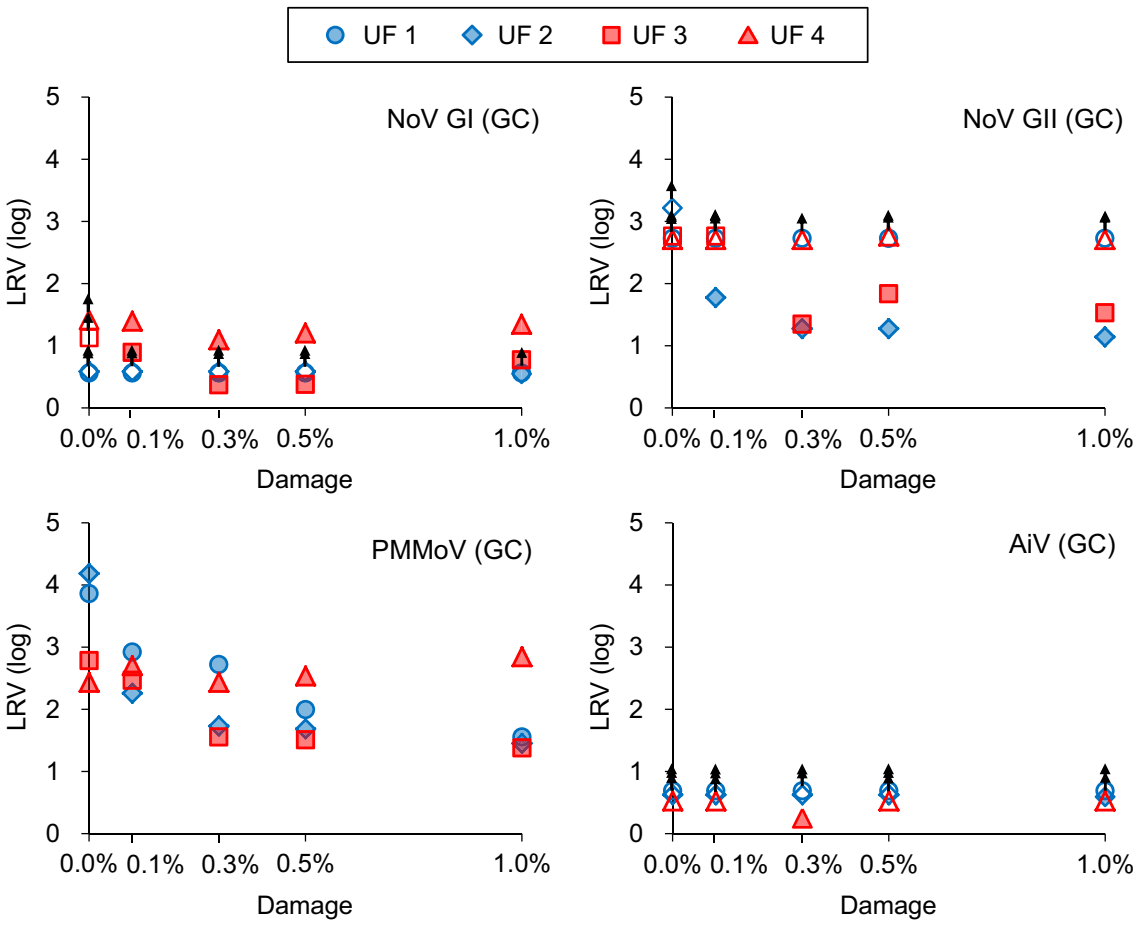

Fig. 2 LRVs of NoV GI and GII, PMMoV, and AiV, which were measured by RT-qPCR ("GC" = genome copy), in the fiber breakage tests using UF 1–4. AiV was not detected in the feed water in the test using UF 3. White plots with the black arrows indicate values below the detection limit, actual LRVs are higher than shown

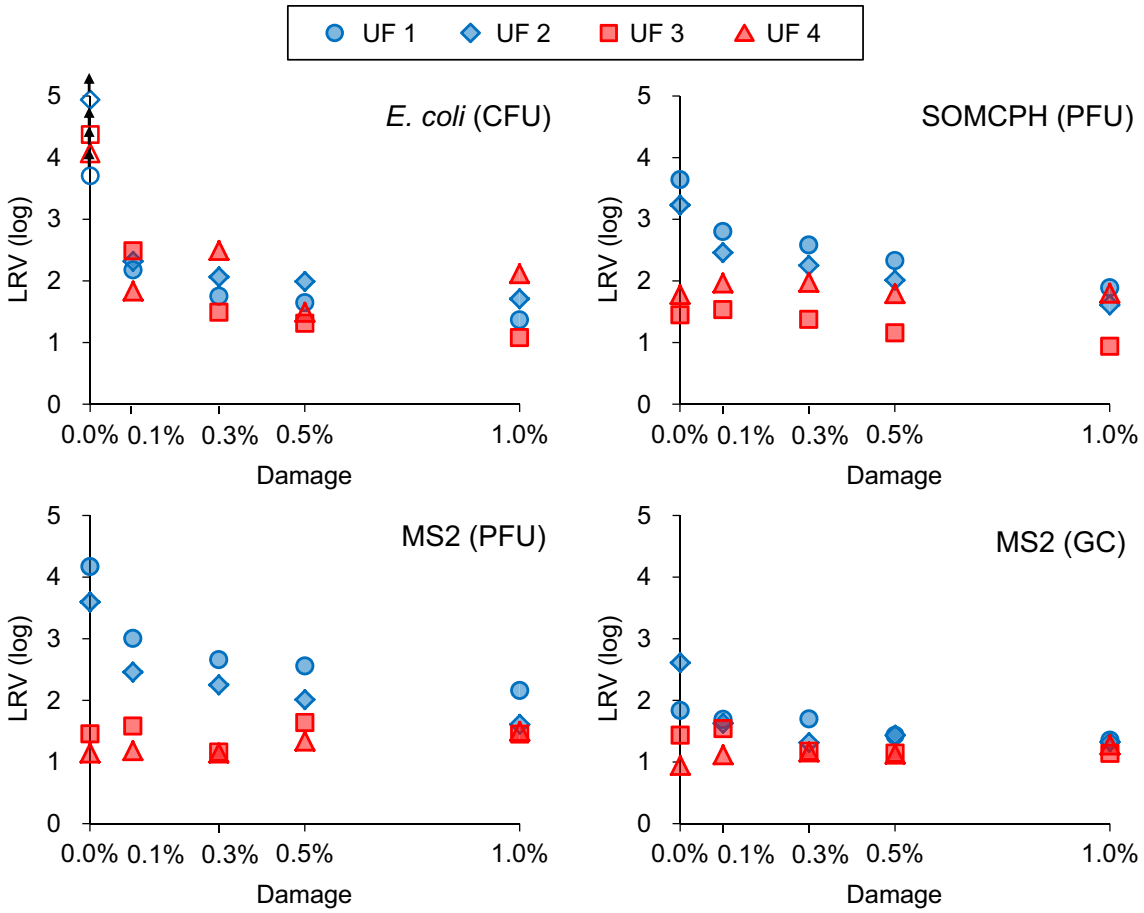

Fig. 3 LRVs of *E. coli*, SOMCPH, and spiked MS2 in the fiber breakage tests using UF 1–4. MS2 was measured by plaque assay ("PFU") and RT-qPCR ("GC" = genome copy). White plots with the black arrows indicate values below the detection limit; hence, actual LRVs are higher than shown

UF 1 and 2 (new UF modules) was also smaller by GC than by PFU.

Effect of LRV of Intact UF Module on LRV Decrease Following Fiber Breakage

The predicted LRVs of MS2, SOMCPH, and PMMoV calculated by Eq. 3 agreed well with the measured LRVs (Fig. 4). In particular, all predicted LRVs of SOMCPH and MS2 determined by plaque assay lay within ± 0.5 $\log_{10}$ unit. Thus, we used Eq. 3 to predict the effect of the LRV of intact UF module ($LRV_i = 1, 2, 3, 4, 5$ $\log_{10}$) on fiber breakage (Fig. 5). When LRV_i was 1 or 2 $\log_{10}$, increased breakage did not significantly decrease LRV, but when it was ≥ 3 $\log_{10}$, it did decrease LRV, increasingly so as LRV_i increased.

Discussion

We assessed the decreases of LRVs of human enteric viruses (NoV GI and GII), viral indicators (PMMoV, AiV, SOMCPH), and a bacterial indicator (*E. coli*) by a sequence of fiber breakages in new and aged UF membranes, and the removal of MS2 as measured by both plaque assay and RT-qPCR, to evaluate the influence of fiber breakage on the removal of microorganisms from reclaimed water.

NoV is the leading cause of gastroenteritis around the world. Secondary effluent, which is used as feed water for wastewater reclamation, contains high concentrations of NoVs, particularly in winter (Katayama et al.

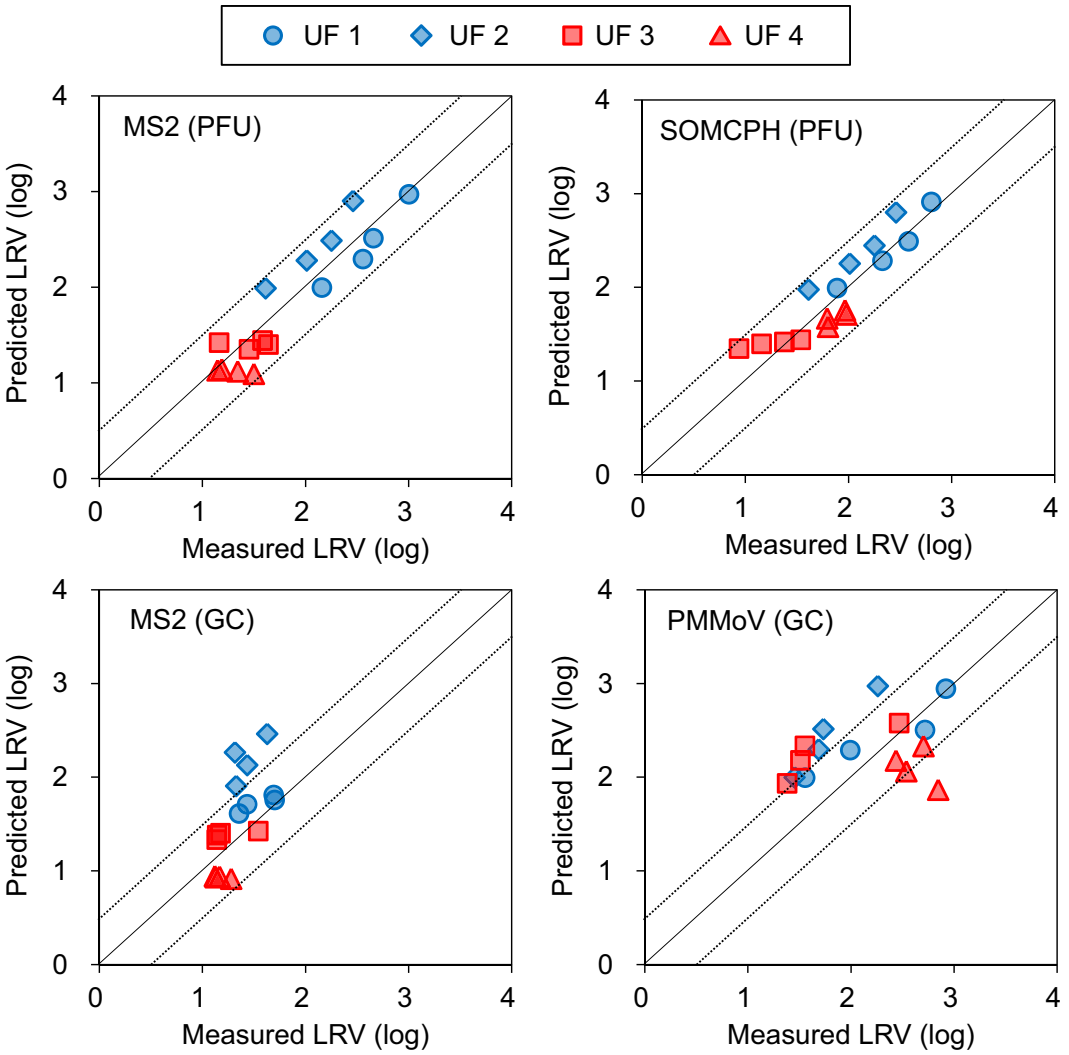

Fig. 4 Comparison of predicted and measured LRVs of spiked MS2, SOMCPH, and PMMoV in fiber breakage tests using UF 1–4. MS2 was measured by plaque assay ("PFU") and RT-qPCR ("GC" = genome copy). The solid line is 1:1, and dotted lines represent ± 0.5 log unit

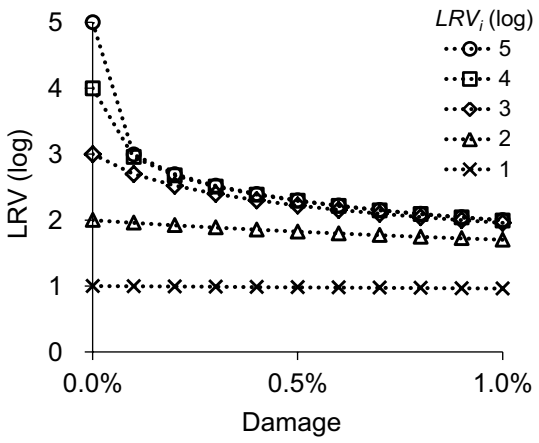

Fig. 5 LRVs predicted by Eq. 3 (N_t = 1000; N_b = 1–10) at assumed values of LRV_i (1, 2, 3, 4, 5 $\log_{10}$) in fiber breakage of UF module

2008; Pérez-Sautu et al. 2012; Lee et al. 2017a). We detected NoVs at high concentrations in secondary effluent (Table 4). Previous studies reported LRVs of NoVs by UF of 3.3 to > 4.5 $\log_{10}$ (Qiu et al. 2015; Yasui et al. 2016). At LRVs of > 4.5, NoVs were not detected in UF-treated samples and were thus adequately removed (Qiu et al. 2015). In our previous study also, NoVs were not detected after UF with LRVs of > 0.5–>1.4 $\log_{10}$ (NoV GI) and > 1.6–>2.5 $\log_{10}$ (NoV GII) (Lee et al. 2017a). Here also, NoVs were not detected in the permeate samples from the intact UF modules, with LRVs of > 0.6–>1.4 $\log_{10}$ (GI) and > 2.7–>3.2 $\log_{10}$ (GII: Fig. 2 and Table S1). These results indicate that the risk of infection by NoVs posed by waters treated by intact UF modules is low. The LRV for NoVs are likely underestimated, since the NoV concentrations in the permeate samples were below the detection limit. Thus, there is a possibility that the actual

risk of infection by NoVs is low. However, NoVs were detected in the permeate samples of UF 2–4 with only 1 fiber breakage out of 1000 (0.1%). This indicates that NoVs can pass through a single broken fiber. LRVs of NoV GI and GII were decreased as breakage increased (0.1–1.0%), with maximum decreases in > 0.7 $\log_{10}$ (GI) and > 2.1 $\log_{10}$ (GII). Thus, fiber breakage in UF modules may increase the risk of infection by NoVs by more than 100 times (> 2.1 $\log_{10}$ reduction of LRV of GII in UF 2: blue open diamond in Fig. 2). These results indicate the need to detect fiber breakage rapidly.

PMMoV and AiV are highly prevalent in surface waters (Hamza et al. 2011; Haramoto et al. 2013; Lodder et al. 2013; Kuroda et al. 2015), wastewater, and treated wastewater (Rosario et al. 2009; Sdiri-Loulizi et al. 2010; Burutarán et al. 2016; Rachmadi et al. 2016; Schmitz et al. 2016; Lee et al. 2017a) with no seasonal change in concentration and thus are suggested as indicators for determining the efficiency of virus removal in drinking water treatment (Asami et al. 2016; Shirasaki et al. 2017, 2018) and water reclamation systems (Kitajima et al. 2014). Here, they were detected in the feed water at rates of 100% and 75%, respectively (Table 4), as similarly reported previously (Kitajima et al. 2014; Lee et al. 2017a). Their LRVs for intact UF modules were 2.4–4.2 $\log_{10}$ (PMMoV) and > 0.5–>0.7 $\log_{10}$ (AiV: Fig. 2 and Table S1), comparable to the values reported in our previous study (2.6–>4.6 $\log_{10}$ and > 1.1–>1.4 $\log_{10}$: Lee et al. 2017a). However, LRVs of PMMoV were decreased by fiber breakages by up to 2.7 $\log_{10}$ (Fig. 2 and Table S1). AiV, which was not detected in the permeate samples from the intact UF modules, was also detected after fiber breakage, with an LRV decrease in > 0.2 $\log_{10}$ (Fig. 2 and Table S1). These results indicate that PMMoV and AiV can also pass through broken fibers.

Escherichia coli, a traditional bacterial indicator, is in general highly removed by UF because of its larger size than the membrane pores and has never been found in UF permeate (Ferrer et al. 2015). We did not detect it in any permeate samples from intact UF modules (LRVs of > 3.7–>4.9 $\log_{10}$: Fig. 3 and Table S2). However, LRVs were reduced in all UF permeate samples by only 1 fiber breakage out of 1000 (0.1%), and its decease in LRV was the greatest of all the targeted microorganisms at 0.1% breakage (> 1.6–>2.7 $\log_{10}$). It further decreased at 1.0% breakage of UF 1–3 (by > 3.3 $\log_{10}$). These results suggest that *E. coli* can pass through broken fibers in spite of its large size (0.5 × 1.0–3.0 μm), and thus protozoan cysts and oocysts of a similar size (3–15 μm), may also pass through a break. It is therefore important to detect fiber breakage rapidly. On the other hand, LRVs of *E. coli* (Fig. 3), NoV GI and GII, and PMMoV (Fig. 2) were not consistently decreased by increasing breakage in UF 4. One possible explanation for this result was clogging of the broken fibers during filtration, as membrane breaches can be plugged by solids adhering to the membrane surface in a membrane bioreactor (Hirani et al. 2014). Further investigation is needed to test this hypothesis for the UF of secondary effluent.

SOMCPH and MS2 are used as viral indicators in UF treatment because their size, structure, and behavior are similar to those of enteric viruses (Langlet et al. 2009; Boudaud et al. 2012; Elhadidy et al. 2013, 2014; Lee et al. 2017a, b). LRVs of SOMCPH and MS2 (PFU) in intact UF modules were similar (Fig. 3). Previous studies also reported that the LRV of SOMCPH by UF is similar to that of MS2 (Elhadidy et al. 2013, 2014), and we previously reported no significant difference between SOMCPH and MS2 in UF (Lee et al. 2018). On the other hand, LRVs of SOMCPH and MS2 (PFU) on intact UF 1 and 2 (3.2–4.2 $\log_{10}$) were higher than those on intact UF 3 and 4 (1.2–1.8 $\log_{10}$: Fig. 3 and Table S2). Furthermore, LRVs of PMMoV on intact UF 1 and 2 were also 1.1–1.8 $\log_{10}$ higher than those on intact UF 3 and 4 (Fig. 2 and Table S1). UF 1 and 2 had been used for only 1–2 weeks, whereas UF 3 and 4 had been used for 4 years (Table 2). Reeve et al. (2016) reported that the median LRV of new UF module (6 months old) was 1 $\log_{10}$ higher than that of aged UF module (6 years old). We previously also reported that the mean LRV of MS2 by new UF module (< 1 year old) was 1.3 $\log_{10}$ higher than that of aged UF module (> 2 years old) (Lee et al. 2017a, 2018). This higher LRV of new UF module might be due to sorption, because new UF module has more surface sites for virus binding than aged UF module. Previous studies showed that virus removal efficiency of UF decreases with the volume of water passed through the membrane owing to saturation of membrane surface sites for virus binding (Van Voorthuizen et al. 2001; Huang et al. 2012; Shirasaki et al. 2017). We investigated MS2 reduction by a 1- to 3-year-old used UF module of the same type as used here at the same pilot plant at WWTP A, and found LRVs (1.5–2.8 $\log_{10}$, $n = 7$, unpublished data) 0.8–2.7 $\log_{10}$ lower than here. Thus, the difference in LRV between the new UF modules (UF 1 and 2) and the aged UF modules (UF 3 and 4) is probably due to the period of use, because the new UF modules have much higher sorption capacity.

Increasing the number of breakages in UF 1 and 2, but not UF 3 and 4, decreased the LRVs of SOMCPH and MS2 (PFU) (Fig. 3 and Table S2). This difference between the new and aged UF modules might be due to the effect of the initial LRV of the intact UF module (LRV_i) on the decrease in LRV caused by increasing breakage: as the assumed LRV_i increased (from 1 to 5 $\log_{10}$), predicted LRV decreased faster with more breakages (Fig. 4). The decrease in the LRVs of SOMCPH and MS2 (PFU) in UF 3 and 4 (1.2–1.8 $\log_{10}$) was much smaller than those in UF 1 and 2 (3.2–4.2 $\log_{10}$: Fig. 3 and Table S2), as predicted by Eq. 3 (Fig. 5). These results suggest that the LRV of intact UF module strongly affects

the decrease in LRV caused by fiber breakage and that a higher LRV_i leads to a greater decrease in LRV on breakage. A higher LRV for a UF module indicates that LRV of each individual fiber is also higher than that of a UF module with a lower LRV UF module. Thus, if one fiber of a high-LRV UF module breaks, the loss of LRV is more affected by the fiber breakage than for one fiber of a low-LRV UF module. This explains why the LRV of *E. coli*, which had the highest LRV of the targeted organisms in intact UF module, decreased the most.

Virus removal or inactivation is investigated by both culture and RT-qPCR methods (Langlet et al. 2009; Furiga et al. 2011; Boudaud et al. 2012). Because RT-qPCR detects viral genomes regardless of infectivity, the concentration includes particles with a broken capsid or free RNA. LRVs of MS2 (GC) by intact UF 1 and 2 were 1.0–2.4 $\log_{10}$ smaller than those of MS2 (PFU), and the LRV decrease in MS2 (GC) caused by increasing breakage was also smaller than that of MS2 (PFU) (Fig. 3 and Table S2). Langlet et al. (2009) reported that LRV by culture was 1.0 $\log_{10}$ higher than that by RT-qPCR, because non-infectious particles are removed less efficiently than infectious ones. As mentioned above, LRVs by the new UF modules (UF 1 and 2) were higher than those by the aged UF modules (UF 3 and 4) because of sorption. Non-infectious particles may be absorbed less efficiently than infectious ones because they have no capsid or a broken one. Thus, LRVs of MS2 (GC) were smaller than those of MS2 (PFU) by intact UF 1 and 2 because RT-qPCR detects non-infectious particles, which pass through the UF membrane without sorption. Furthermore, these lower LRVs of MS2 (GC) in intact UF 1 and 2 lead to the lower LRV decrease as breakages increase than indicated by MS2 (PFU).

The predicted LRVs agreed well with the measured LRVs of SOMCPH and MS2 determined by plaque assay (Fig. 4). On the other hand, the predicted LRVs disagreed with the measured LRVs of MS2 and PMMoV determined by RT-qPCR. As RT-qPCR assay detects both infectious and non-infectious viruses, the behavior of non-infectious viruses may differ from that of infectious viruses when a membrane fiber breaks. However, Eq. 3 could be useful for predicting LRV following fiber breaks because of the good fit with LRVs of infectious phages, which were determined by plaque assay.

We investigated the effect of fiber breakages using only one UF module (1000 fibers), but full-scale UF plants use > 100 modules (> 100,000 fibers). Hence, if we assume 1–10 fiber breakages at plant A with 10 UF modules (10,000 fibers) and at plant B with 100 modules (100,000 fibers), Eq. 3 predicts the LRV decrease as < 3 $\log_{10}$ at plant A and < 4 $\log_{10}$ at plant B, which have no notable effect (Fig. S1). Therefore, 1–10 fiber breakages at full-scale plants with 100 UF modules are unlikely to affect LRVs of microorganisms at LRV < 4 $\log_{10}$. However, careful consideration must be given to LRV decreases in microorganisms such as *E. coli* and NoV, which were not detected in any feed samples (LRV > 4 $\log_{10}$), by fiber breakage.

Conclusion

All indigenous microorganisms targeted in this study were detected in feed waters at the pilot-scale UF plants. NoVs, AiV, and *E. coli* were not detected in any permeate samples of intact UF modules, but were detected in those of damaged UF modules. LRVs of all target microorganisms decreased as breakages of new UF modules increased, by up to 3.3 $\log_{10}$ (*E. coli*). LRVs of SOMCPH and MS2 in new UF modules, but not aged UF modules, decreased significantly as breakage increased. Intact new UF modules gave higher LRVs than intact aged UF modules owing to sorption. When the initial LRV of intact UF module was assumed to be 1 or 2 $\log_{10}$, increased breakage did not significantly decrease the predicted LRV, but when it was ≥ 3 $\log_{10}$, the predicted LRV decreased substantially. These results suggest that the initial LRV of intact UF module determined the LRV decrease, and that microorganisms reduced more by UF are affected more by fiber breakage.

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