Feasibility of in-line coagulation as a pretreatment for ceramic microfiltration to remove viruses

Nobutaka Shirasaki, Taku Matsushita, Yoshihiko Matsui, Makoto Kobuke and Koichi Ohno

ABSTRACT

The feasibility of in-line coagulation as a pretreatment for ceramic microfiltration (MF) was verified by comparing its efficiency in the removal of viruses with that of the traditional mechanical mixing approach for coagulation, and by examining the effect of coagulant dose and coagulation time on virus removal. The in-line coagulation–ceramic MF system efficiently removed bacteriophage Qβ and MS2: removal ratios were >8.2 log for infectious viruses and >5.4 log for total (infectious + inactivated) virus particles. These values were similar to those of the mechanical coagulation–ceramic MF system. The in-line coagulation system has potential as a useful pretreatment for the removal of viruses as an alternative to the mechanical mixing system, because the former efficiently removes viruses and has a smaller footprint in treatment plants. For the in-line coagulation–ceramic MF system, a coagulant dose of 1.08 mg-Al/L and a coagulation time of 1 min were required to achieve a high level of virus removal. Infectious Qβ and MS2 were removed to similar levels by the two precoagulation methods tested, but the removal of total MS2 particles was higher than that of Qβ particles, possibly because of the selective interaction with the cake layer.

Key words | bacteriophages, ceramic microfiltration, in-line coagulation, virus inactivation, virus removal

Nobutaka Shirasaki (corresponding author)
Taku Matsushita
Yoshihiko Matsui
Makoto Kobuke
Koichi Ohno
Division of Built Environment,
Graduate School of Engineering,
Hokkaido University,
N13W8,
Sapporo 060-8628,
Japan
Tel.: +81-11-706-7281
Fax: +81-11-706-7279
E-mail: nobutaka@eng.hokudai.ac.jp

INTRODUCTION

Membrane filtration processes using microfiltration (MF) and ultrafiltration (UF) membranes have been widely used to produce drinking water. One of the primary reasons for the increasing use of membrane filtration is that it can be used to remove pathogenic microorganisms such as *Cryptosporidium* and *Giardia*. Complete removal of *Cryptosporidium parvum* oocysts and *Giardia muris* cysts was achieved by direct MF/UF membranes (Jacangelo *et al.* 1995). By comparison, because viruses are the smallest pathogenic microorganisms among the causative agents of waterborne disease, varying levels of virus removal have been reported for direct MF (<0.5 to >6 log)/UF (0.4 to >6 log) processes (Jacangelo *et al.* 1995; Urase *et al.* 1996; Otaki *et al.* 1998; Hu *et al.* 2003; Arkhangelsky & Gitis 2008;

Langlet *et al.* 2009). The 4-log virus removal required by the US Environmental Protection Agency (USEPA 2001) is regularly not satisfied by MF/UF processes alone, particularly direct MF processes.

Recently, coagulation as a pretreatment for membrane filtration has become attractive because it is expected to improve removal of organic compounds (Yuasa 1998; Kim *et al.* 2006; Choi *et al.* 2008; Kimura *et al.* 2008) and to mitigate membrane fouling (improve flux decline) (Kunikane *et al.* 1995; Judd & Hillis 2001; Oh & Lee 2005; Cho *et al.* 2006; Kim *et al.* 2006; Choi *et al.* 2008; Kimura *et al.* 2008). The combination of coagulation and MF was shown to achieve higher levels of virus removal than that by MF alone (Zhu *et al.* 2005; Fiksdal & Leiknes 2006).

Our group has also reported the usefulness of a coagulation–ceramic MF system for virus removal (Matsui et al. 2003a; Matsushita et al. 2005): a >6-log removal of viruses was achieved by this system with an aluminium coagulant.

In the coagulation–MF processes, coagulation conditions, including coagulant dose and coagulation time, affect membrane performance. Although many studies have focused mostly on the influence of membrane fouling (e.g. Lee et al. 2000; Judd & Hillis 2001; Oh & Lee 2005; Cho et al. 2006; Kimura et al. 2008), few researchers have reported the effect of coagulant dose (Matsui et al. 2003a; Matsushita et al. 2005; Zhu et al. 2005; Fiksdal & Leiknes 2006) and coagulation time (Matsushita et al. 2005) on virus removal. Zhu et al. (2005) combined polyvinylidene fluoride MF with jar coagulation (without settling) and reported a dramatic increase in virus removal with an increase in the dose of ferric chloride. Matsushita et al. (2005) also reported that coagulant dose strongly affects virus removal: only 2.8-log removal was achieved with 0.54 mg-Al/L polyaluminium chloride (PACl) in the in-line coagulation–ceramic MF process, whereas 6.4-log and 7.4-log removal was achieved with 1.08 and 1.62 mg-Al/L PACl, respectively. Matsushita et al. (2005) also revealed that a longer coagulation time provides a greater reduction in virus level.

Coagulation as a pretreatment for membrane filtration can be introduced by mechanical mixing or in-line mixing. Mechanical mixing is widely employed in the traditional treatment of drinking water consisting of coagulation, flocculation, sedimentation and rapid sand filtration processes. For these processes, rapid mixing and slow mixing tanks are installed in the system to allow colloids in water, following addition of the coagulant, to coagulate and flocculate. The MF process can be an alternative to the rapid sand filtration process as a physical barrier. Three applications are available for mechanical mixing in coagulation pretreatment. Raw water can be treated with the coagulant in the rapid mixing tank and then subjected to MF without slow mixing and sedimentation. Alternatively, raw water can be treated in the rapid mixing tank and then passed through a slow mixing tank only or through a slow mixing tank and then a sedimentation tank before MF. Treatment of the raw water with coagulant in the rapid mixing tank only is often applied to the ceramic MF process (Yonekawa et al. 2004), whereas the application of a slow mixing tank and a sedimentation tank is often used together with organic membranes (Bakersfield Water Treatment Plant, California, http://www.water-technology.net/projects/bakersfield/, accessed 4 March 2009; Columbia Heights Filtration Plant, Minneapolis, http://www.water-technology.net/projects/columbia/, accessed 4 March 2009).

As an alternative procedure, in-line coagulation as a pretreatment for MF has been tested in bench-scale and pilot-scale experiments (Judd & Hillis 2001; Matsui et al. 2003a; Matsushita et al. 2005; Oh & Lee 2005; Cho et al. 2006; Meyn et al. 2008) because of its advantages over mechanical mixing, including reduction of coagulant dose, coagulation time and energy consumption (Oh & Lee 2005; Meyn et al. 2008). Comparisons of the operation performance of these types of mechanical mixing are limited. The efficiency of dissolved organic carbon (DOC) removal has been compared among three different types of mechanical mixing for coagulation with 6 mg-Fe/L iron chloride dosing including in-line mixing (hydraulic retention time (HRT), 45 s), one-stage mechanical rapid mixing (HRT, 6.8 min), and two-stage flocculation with mechanical rapid mixing and slow mixing (total HRT, 20 min) for the ceramic MF process, and the efficiency was found to be almost the same, regardless of the mixing type (Meyn et al. 2008). The effect on virus removal has not been investigated, except in a previous study by our group (Matsui et al. 2003a).

The virus removal performance of membrane filtration processes, including the coagulation–MF process, is frequently evaluated by using bacteriophages (i.e. viruses that infect bacteria) as indicators of enteric viruses (Jacangelo et al. 1995; Urase et al. 1996; Otaki et al. 1998; Hu et al. 2003; Matsui et al. 2003a; Matsushita et al. 2005; Zhu et al. 2005; Fiksdal & Leiknes 2006; Arkhangelsky & Gitis 2008; Langlet et al. 2009; Shirasaki et al. 2009a). The plaque-forming unit (PFU) method is commonly used for quantification of bacteriophages, because it measures the concentration of infectious viruses. However, the removal of infectious viruses by the coagulation–MF process is due not only to physical removal during the membrane separation process but also to the virucidal activity of the aluminium coagulant (Matsui et al. 2003b; Matsushita et al. 2004; Shirasaki et al. 2009b) during the coagulation pretreatment.

In a previous study, our group applied the PFU method together with the polymerase chain reaction (PCR) method

to measure the concentration of infectious viruses as well as inactivated viruses, to evaluate the performance of the coagulation–MF process in removing infectious viruses and inactivated viruses (Shirasaki *et al.* 2009*a*). We found a difference between total (infectious + inactivated) and infectious virus concentrations just before MF, indicating inactivation of viruses during coagulation. Although the mechanisms underlying the virucidal activity of the aluminium coagulant remain unclear, inactivated viruses in the MF permeate might recover their infectivity in the water distribution system. Virus removal performances evaluated by the PFU method might underestimate the risk of infection because this method cannot count the inactivated viruses that pass into the MF permeate.

The effects of coagulation conditions on the performance of infectious and inactivated virus removal have not been investigated. By comparison, the effects of the different mixing methods (in-line mixing vs. mechanical mixing) (Matsui *et al.* 2003*a*), coagulant dose (Matsui *et al.* 2003*a*; Matsushita *et al.* 2005; Zhu *et al.* 2005; Fiksdal & Leiknes 2006) and coagulation time (Matsushita *et al.* 2005) on the removal of infectious viruses have been widely investigated by using the PFU method alone.

Our objective in the present study was to verify the feasibility of in-line coagulation as a pretreatment for ceramic MF by comparing its efficiency for virus removal with that of the traditional mechanical mixing approach for coagulation, as well as by examining the effect of coagulant dose and coagulation time on virus removal. The efficiency of virus removal in these experiments was assessed by using the PFU method, to measure the concentration of infectious bacteriophage, and real-time reverse transcription–polymerase chain reaction (RT-PCR) method, to measure the concentration of total bacteriophage.

MATERIALS AND METHODS

Source water, coagulant and MF membranes

River water was sampled from the Toyohira River (Sapporo, Japan) on 12 June 2008 and was subjected to water quality analyses (Table 1). PACl (250A; 10.5% Al_2O_3, relative density 1.2 at 20°C; Taki Chemical Co., Ltd, Hyogo, Japan)

Table 1 │ Water quality of the Toyohira River

pH	7.2
DOC (mg/L)	0.76
OD260 (cm^{-1})	0.019
Turbidity (NTU)	0.63
Alkalinity (mg-CaCO$_3$/L)	17.2

was used for the coagulation process. The membrane used was a monolithic ceramic MF module installed in a stainless-steel casing (61-channel tubular; nominal pore size 0.1 μm, effective filtration area 0.048 m^2, membrane diameter 0.03 m, membrane length 0.1 m; NGK Insulators, Ltd, Nagoya, Japan).

Bacteriophages

The F-specific RNA bacteriophages Qβ (NBRC 20012) and MS2 (NBRC 102619) were obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). The bacteriophages Qβ (Urase *et al.* 1996; Otaki *et al.* 1998; Matsui *et al.* 2003*a*; Matsushita *et al.* 2005; Langlet *et al.* 2009; Shirasaki *et al.* 2009*a*) and MS2 (Jacangelo *et al.* 1995; Hu *et al.* 2003; Zhu *et al.* 2005; Fiksdal & Leiknes 2006; Arkhangelsky & Gitis 2008; Langlet *et al.* 2009; Shirasaki *et al.* 2009*a*) are widely used as surrogates for pathogenic waterborne viruses because of their morphological similarities to hepatitis A viruses and polioviruses, which need to be removed by the treatment of drinking water.

Qβ is the prototype member of the genus *Allolevivirus* in the family Leviviridae, and MS2 is the prototype member of the genus *Levivirus* in the family Leviviridae. The Qβ and MS2 genomes comprise a single molecule of linear positive-sense, single-stranded RNA encapsulated in an icosahedral protein capsid with a diameter of 24–26 nm (The Universal Virus Database of the International Committee on Taxonomy of Viruses, http://phene.cpmc.columbia.edu/index.htm, accessed 17 April 2008). Each bacteriophage was propagated for 22–24 h at 37°C in *Escherichia coli* (NBRC 13965) obtained from NBRC. The bacteriophage culture solution was centrifuged (2,000 × *g*, 10 min) and then passed through a membrane filter (pore size 0.45 μm, hydrophilic cellulose acetate; Dismic-25cs, Toyo Roshi Kaisha, Ltd, Tokyo, Japan).

To prepare the bacteriophage stock solution, the filtrate was purified by using a centrifugal filter device comprising a regenerated cellulose membrane with a molecular weight cut off of 100,000 (Amicon Ultra-15, Millipore Corp., Billerica, Massachusetts).

In-line coagulation–ceramic MF experiments

The setup for the in-line coagulation–ceramic MF experiments is schematically depicted in Figure 1. The river water, placed in the raw water tank, was spiked with either Qβ or MS2 at approximately 10^8 PFU/mL. Throughout the experiments, the raw water was mixed constantly with an impeller stirrer. The raw water was fed into the system at a constant flow rate $(83.3 \, L/(m^2 h) = 2.0 \, m/d)$ by a peristaltic pump. To maintain the MF permeate at pH 6.8, hydrochloric acid or sodium hydroxide was added to the water before it reached the first in-line static mixer (HRT 1.8 s; 1/4-N40-172-0, Noritake Co., Ltd, Nagoya, Japan).

PACl was injected after the first in-line static mixer and before the second in-line static mixer at a constant dose rate (0.54, 1.08 or 1.62 mg-Al/L). To obtain the three different coagulation times, the in-line static mixer (G value 260/s, HRT 1.8 s), and a combination of the in-line static mixer and a subsequent Tygon® tube reactor (total HRT 1 or 5 min), were used as the second in-line static mixer. The total HRT was controlled by the length of the Tygon® tube reactor. After the PACl had been mixed in, the water was

fed into the ceramic MF module in dead-end mode. Filtration was performed for 4 h without any backwashing. Bacteriophage concentrations in the raw water tank and in the MF permeate were measured every hour.

Mechanical coagulation–ceramic MF experiments

The setup for mechanical coagulation–ceramic MF experiments is schematically depicted in Figure 2. The river water, placed in the raw water tank, was spiked with either Qβ or MS2 at approximately 10^8 PFU/mL. Throughout the experiments, the raw water was mixed constantly with an impeller stirrer. The raw water was fed into the system at a constant flow rate (100 mL/min) by a peristaltic pump. To maintain the MF permeate at pH 6.8, hydrochloric acid or sodium hydroxide was added to the water before it reached the first in-line static mixer (HRT 1.2 s). pH-adjusted raw water was introduced and PACl was injected into the rapid mixing chamber with an impeller stirrer (G value 200/s, 109 rpm; hydraulic retention time 5 min) at a constant dose rate (1.08 mg-Al/L).

After the PACl had been mixed in, the water was passed through five chambers for slow mixing (G value 20/s, 23 rpm; HRT 5 min × 5 chambers) and then through a rectangular settler for settling of the aluminium floc particles (HRT 20 min). At the end of the rapid mixing or settling, the water was fed into the ceramic MF module in dead-end mode at a constant flow rate 67 mL/min, which
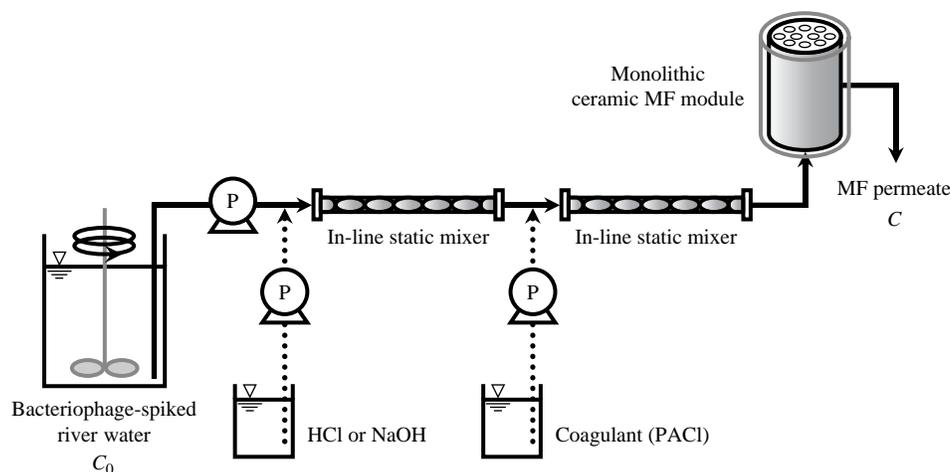

Figure 1 │ In-line coagulation–ceramic MF system. C_0 and C are the bacteriophage concentrations (PFU or particles/mL) in the raw water tank and the MF permeate, respectively, at each sampling time.
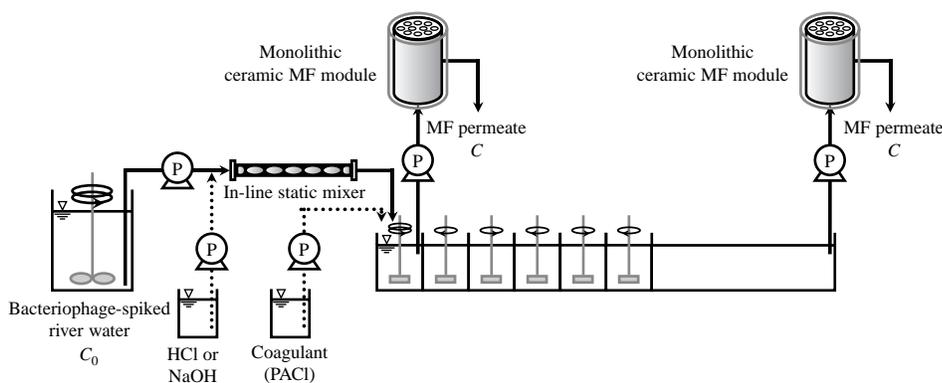
Figure 2 | Mechanical coagulation–ceramic MF system. C_0 and C are the bacteriophage concentrations (PFU or particles/mL) in the raw water tank and the MF permeate, respectively, at each sampling time.

was equivalent to $83.3\,L/(m^2\,h)$. Filtration was performed for 4 h without any backwashing. Bacteriophage concentrations in the raw water tank and in the MF permeate were measured every hour.

Bacteriophage assay

PFU method

The infectious bacteriophages were enumerated according to the double-layer method (Adams 1959) by using the bacterial host *E. coli* (NBRC 13965).

Serially diluted raw water or MF permeate (1 mL) was poured onto a solid bottom agar plate followed by 0.3 mL of host *E. coli* culture mixed with 3 mL of molten top agar. The plates were incubated for 16–24 h at 37°C. To measure the concentration of infectious bacteriophage in the water samples, we calculated the average plaque counts of triplicate plates prepared from one sample on plates with 30 to 300 PFU, which we considered a countable number of plaques, and determined the PFU/mL.

For quantification of low infectious bacteriophage concentrations (i.e. < 30 PFU/mL) in the MF permeate, 50 mL of MF permeate was mixed with 5 mL of bacterial host *E. coli* culture and 50 mL molten agar, and the mixture was then poured into 10 plates (without bottom agar). The plates were incubated for 16–24 h at 37°C. We calculated the PFU/mL by dividing the total plaque counts for the 10 plates by the sample volume (50 mL).

Real-time RT-PCR method

Viral RNA of bacteriophages was quantified by real-time RT-PCR method. Real-time RT-PCR method detects the virus genome and therefore detects viruses regardless of their infectivity. We defined the concentration measured by real-time RT-PCR method as the total bacteriophage concentration. For quantification of bacteriophages in the raw water and MF permeate, viral RNA was extracted from 200 μL samples with a QIAamp MinElute Virus Spin Kit (Qiagen K.K., Tokyo, Japan) to obtain a final volume of 20 μL. The viral RNA was then subjected to RT reaction by using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems Japan Ltd, Tokyo, Japan). RT reaction was conducted at 25°C for 10 min, 37°C for 120 min, and 85°C for 5 s, followed by cooling to 4°C in a thermal cycler (Thermal Cycler Dice Model TP600, Takara Bio Inc., Shiga, Japan). The resultant cDNA was then amplified by using a TaqMan Universal PCR Master Mix with UNG (Applied Biosystems Japan Ltd), 400 nM of each primer (HQ-SEQ grade, Takara Bio Inc.), and 250 nM of TaqMan probe (Applied Biosystems Japan Ltd). The oligonucleotide sequences of the primers and the probes are listed in Table 2. Amplification was conducted at 50°C for 2 min, 95°C for 10 min, and then for 40 cycles at 95°C for 15 s and 60°C for 1 min in an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems Japan Ltd).

We constructed a standard curve for real-time RT-PCR method based on the relationship between the infectious

Table 2 | Oligonucleotide sequences of the primers and the probes used in real-time RT-PCR quantification of Qβ and MS2

Viruses		Oligonucleotide sequences	Positions	References
Qβ	Forward primer	5′-TCA AGC CGT GAT AGT CGT TCC TC-3′	49–71	Katayama et al. (2002)
	Reverse primer	5′-AAT CGT TGG CAA TGG AAA GTG C-3′	187–208	
	TaqMan probe	5′-CGA GCC GCG AAC ACA AGA ATT GA-3′	147–169	
MS2	Forward primer	5′-GTC GCG GTA ATT GGC GC-3′	632–648	O'Connell et al. (2006)
	Reverse primer	5′-GGC CAC GTG TTT TGA TCG A-3′	690–708	
	TaqMan probe	5′-AGG CGC TCC GCT ACC TTG CCC T-3′	650–671	

bacteriophage concentration (PFU/mL) of freshly prepared bacteriophage stock solution, assumed not to contain inactivated bacteriophages, and the number of PCR amplification cycles.

RESULTS AND DISCUSSION

Infectious bacteriophage removal by the in-line coagulation–ceramic MF system

Figure 3 shows the effect of coagulant dose and coagulation time on the removal of infectious bacteriophage, assessed by the PFU method, by the in-line coagulation–ceramic MF system. Because the diameters of Qβ and MS2 were smaller than the nominal pore size of the ceramic MF membrane (0.1 μm), there was no removal of these infectious bacteriophages in the absence of coagulation pretreatment (data not shown). Other researchers also have reported
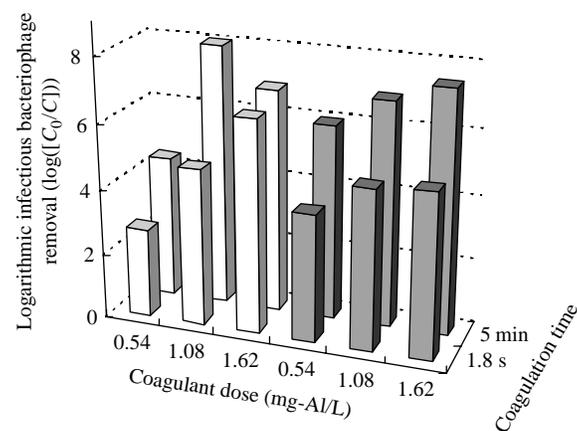

Figure 3 | Effect of coagulant dose and coagulation time on the filtration time-averaged infectious bacteriophage removal in the in-line coagulation–ceramic MF system. White and shaded columns represent Qβ and MS2, respectively.

an insufficient removal ratio for infectious Qβ and MS2 by MF in the absence of coagulation pretreatment: 1–2 log for Qβ (Urase et al. 1996) and <1 log for MS2 (Jacangelo et al. 1995).

By comparison, in-line coagulation pretreatment improved the removal ratios ($\log[C_0/C]$) for infectious Qβ and MS2 by MF (Figure 3) because these phages were negatively charged at pH 6.8 (Shirasaki et al. 2009a) and became adsorbed to/entrapped by the positively charged aluminium floc particles (amorphous aluminium hydroxide, Al[OH]$_3$) generated during coagulation pretreatment. The aluminium floc particles exceeded the pore size of the ceramic MF membrane and were removed during the filtration process. More than 4-log removal was achieved for both bacteriophages, except at a coagulant dose of 0.54 mg-Al/L and a coagulation time of 1.8 s.

According to the USEPA National Primary Drinking Water Standards, 4-log removal or inactivation of enteric viruses from source water is required by filtration, disinfection or a combination of these technologies (USEPA 2001). Our hybrid system successfully met this requirement with a sufficient coagulant dose (≥1.08 mg-Al/L). Some researchers have also reported the usefulness of precoagulation to improve the removal of viruses by MF (Matsui et al. 2003a; Matsushita et al. 2005; Zhu et al. 2005; Fiksdal & Leiknes 2006). MF alone does not remove viruses, but the coagulation–MF system is effective in removing infectious viruses: 6–7-log for Qβ (Matsui et al. 2003a) and 6.7 to >7.5-log for MS2 (Fiksdal & Leiknes 2006).

An increase in the coagulant dose reduced the number of infectious bacteriophages (Figure 3). The time-averaged reduction in infectious Qβ and MS2 increased from 2.7 to 6.5-log and from 3.9 to 5.0-log, respectively, with an increase in the coagulant dose (0.54 to 1.62 mg-Al/L)

at a coagulation time of 1.8 s. The same positive effect of coagulation has been reported in other studies (Matsui *et al.* 2003*a*; Matsushita *et al.* 2005). This effect was most likely due to an increase in floc size with increasing coagulant dose (Judd & Hillis 2001). Increasing the coagulant dose from 0.54 to 1.08 mg-Al/L increased the size of aluminium floc particles with adsorbed/entrapped bacteriophages, and subsequently reduced the number of aluminium floc particles that passed through a ceramic MF membrane.

An increase in coagulation time also reduced the number of infectious Qβ and MS2. An extension of the coagulation time from 1.8 s to 5 min increased the removal ratios for Qβ and MS2 at any coagulant dose, most likely because the floc size during the coagulation process increased with time. Our results indicated that a coagulation time of 1.8 s was insufficient to achieve a high removal ratio for infectious phages.

Overall, there was no difference in the removal ratio between infectious Qβ and MS2 when the system was operated with a sufficient coagulant dose of ≥ 1.08 mg-Al/L and at a coagulation time of 5 min. This result was consistent with the findings of our previous study (Shirasaki *et al.* 2009*a*). Under these conditions, a removal ratio of more than 6.9-log, based on time-averaged reduction, was achieved for Qβ and MS2 by the in-line coagulation–ceramic MF system. This removal ratio was almost the same as or higher than the removal ratios obtained with direct UF (0.4 to >6 log) (Jacangelo *et al.* 1995; Urase *et al.* 1996; Otaki *et al.* 1998; Hu *et al.* 2003; Arkhangelsky & Gitis 2008) and direct nanofiltration (NF) (1.9 to >6 log) (Urase *et al.* 1996; Otaki *et al.* 1998; Hu *et al.* 2003) processes. Thus, the in-line coagulation–ceramic MF system is a potential alternative to UF or NF processes for the removal of infectious viruses. Our results also demonstrated that a high removal ratio could be achieved with effective control of coagulant dose and coagulation time. We propose that a PACl dose of 1.08 mg-Al/L is required to achieve a high removal ratio of infectious viruses in the present in-line coagulation–ceramic MF system.

Total bacteriophage removal by the in-line coagulation–ceramic MF system

Figure 4 shows the effect of coagulant dose and coagulation time on total bacteriophage removal, assessed by real-time
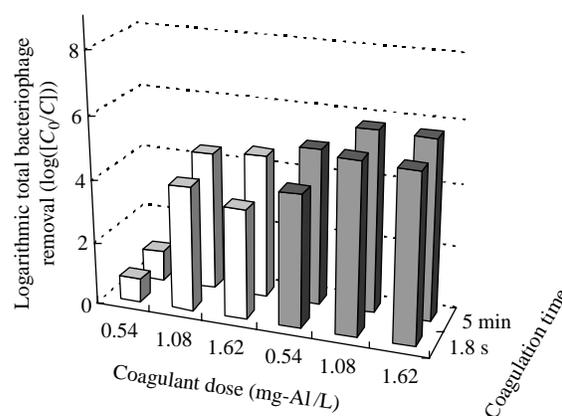

Figure 4 | Effect of coagulant dose and coagulation time on the filtration time-averaged total bacteriophage removal in the in-line coagulation–ceramic MF system. White and shaded columns represent Qβ and MS2, respectively.

RT-PCR method, by the in-line coagulation–ceramic MF system. A low removal ratio (<1.0-log) was observed for total Qβ with a coagulant dose of 0.54 mg-Al/L, but a high removal ratio of more than 4-log was achieved by increasing the coagulant dose from 0.54 to 1.08 mg-Al/L. In addition, extension of the coagulation time from 1.8 s to 5 min increased the removal ratio for total Qβ from 4.0-log to 4.4-log at a coagulant dose of 1.08 mg-Al/L. Thus, both coagulant dose and coagulation time were important factors dominating the removal performance not only of infectious Qβ but also of total Qβ in the in-line coagulation–ceramic MF system.

By comparison, our in-line coagulation–ceramic MF system achieved a high removal ratio of >4-log for total MS2 at all coagulation conditions. Although particle diameters and electrophoretic mobilities were almost the same for Qβ and MS2 (Shirasaki *et al.* 2009*a*), a marked difference in removal ratio was observed between total Qβ and MS2. The removal ratio for total Qβ was lower than that of total MS2 under all coagulation conditions. This difference was most likely the result of differences between the interactions of Qβ and MS2 with the aluminium floc particles accumulated on the membrane surface as a cake layer. It is possible that MS2 has a higher affinity for the cake layer than does Qβ (Shirasaki *et al.* 2009*a*).

Abbaszadegan *et al.* (2007) and Mayer *et al.* (2008) reported that the removal ratio for MS2 was lower than that of adenoviruses, feline caliciviruses, coxsackieviruses, echoviruses and polioviruses by an enhanced coagulation

process using ferric chloride, and these researchers concluded that MS2 was an appropriate surrogate for enteric viruses. In contrast, our results suggest that Qβ may be a more appropriate surrogate than MS2, because we found that Qβ was more difficult to remove than MS2 by our in-line coagulation–ceramic MF system. Langlet *et al.* (2009) evaluated the efficiency of virus removal by direct MF and UF processes, also by using real-time RT-PCR method, and, consistent with our findings, their results demonstrated that the removal ratio for Qβ was lower than that of MS2; they concluded that Qβ was a better candidate than MS2 for characterizing membrane virus removal. We believe that Qβ, rather than MS2, has the potential to become a conservative surrogate for the evaluation of virus removal performance by drinking water treatment processes, including coagulation–MF processes.

The removal ratios of infectious Qβ and MS2 (Figure 3) were higher than those for total Qβ and MS2 (Figure 4). This result indicates that a large proportion of the bacteriophage population was inactivated by the coagulation–MF process. Our group previously reported that Qβ and MS2 were inactivated during the coagulation process and that Qβ was more sensitive to the virucidal activity of PACl than MS2 was (Matsui *et al.* 2003*b*; Matsushita *et al.* 2004; Shirasaki *et al.* 2009*b*). The removal efficiency of total Qβ is greater than that of MS2, but because of differences between these two bacteriophages in their sensitivity to the virucidal activity of PACl, the removal ratio of infectious Qβ and MS2 becomes almost the same.

In summary, the time-averaged removal ratios for total Qβ and MS2 were 4.4-log and 5.8-log, respectively, at a coagulant dose of 1.08 mg-Al/L and at a coagulation time of 5 min. These values were almost the same as or higher than the removal ratios by the direct UF process (1.5 to >6 log) (Langlet *et al.* 2009). Thus, the in-line coagulation–ceramic MF system is a potential alternative to the UF process for the removal of total viruses as well. From our observations, we propose that a PACl dose of 1.08 mg-Al/L is required to achieve a high removal ratio not only of infectious viruses but also of total viruses in the present in-line coagulation–ceramic MF system.

Comparison of removal performance between the in-line and mechanical coagulation processes

As described above, a PACl dose of 1.08 mg-Al/L was required to achieve high removal ratios for both infectious and total bacteriophages in the present in-line coagulation–ceramic MF system. To verify the feasibility of in-line coagulation as a pretreatment for the ceramic MF process, we compared the removal performances of Qβ and MS2 by in-line mixing and the traditional mechanical mixing approach at the same PACl dose (1.08 mg-Al/L).

Figure 5 shows a comparison of virus removal performance between the in-line and the mechanical coagulation–ceramic MF systems. Mechanical rapid mixing followed by MF achieved high removal ratios of >8.2-log for infectious and >4.3-log for total phage for both Qβ and MS2.
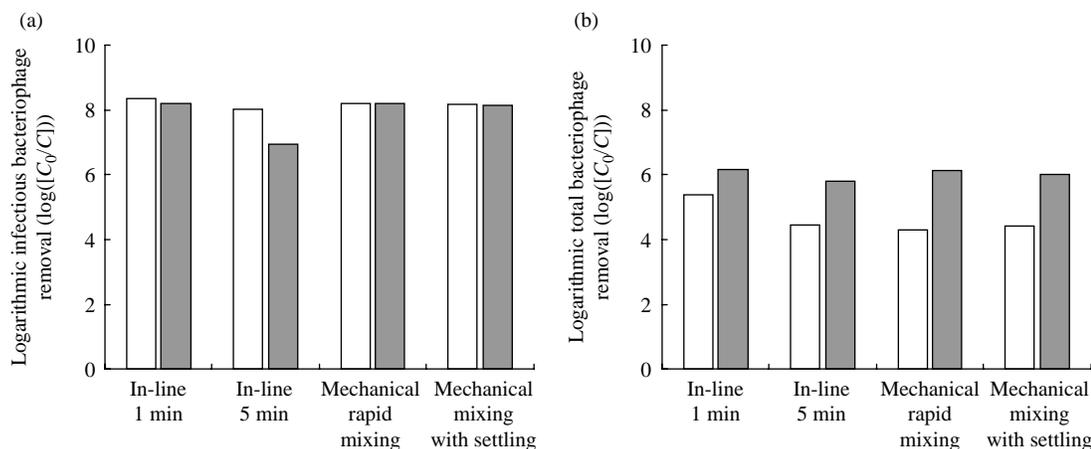

Figure 5 │ Effect of mixing type of coagulation pretreatment on the removal of infectious bacteriophage (a) and total bacteriophage (b). White and shaded columns represent Qβ and MS2, respectively. PACl dosage was 1.08 mg-Al/L.

Our result for infectious Qβ removal agreed with that from the previous study (approximately 7.5-log) (Matsui *et al.* 2003*a*). Removal ratios did not improve even when slow mixing and settling processes were incorporated between the rapid mixing and MF processes. This result indicates that 5 min of rapid mixing was sufficient to achieve high removal ratios for infectious and total bacteriophage in the coagulation–ceramic MF system with mechanical mixing. Additional slow mixing and settling processes are not necessary for virus removal, enabling the actual treatment plant to be compact.

The high removal ratio for Qβ and MS2 achieved with the mechanical mixing system was similar to that achieved with equivalent coagulation time of 5 min in the in-line mixing system, demonstrating that in-line coagulation has potential as a pretreatment for MF giving high virus removal as an alternative to the mechanical mixing system. In addition, even when the coagulation time in the in-line mixing system was reduced to one-fifth (i.e. 1 min), high virus removal ratios of >8.2-log for infectious and >5.4-log for total bacteriophage, were still achieved (Figure 5). This means that 1 min of coagulation time is enough to achieve a high level of virus removal in the present in-line coagulation–ceramic MF system, leading to a reduction in the footprint required for the HRT in the actual treatment plant.

The removal performances for the two bacteriophages were similar for all tested mixing procedures, although the characteristics of aluminium floc particles (i.e. size, fractal dimension, and so on) fed into the ceramic MF module most likely differed depending on the mixing type. One possible explanation is that, during rapid mixing, regardless of mixing type, most of the bacteriophage population was adsorbed to/entrapped by aluminium floc particles that were larger than the pore size of the MF membrane and would thus be removed by the MF process. However, the small proportion of the bacteriophage population adsorbed to/entrapped by aluminium floc particles smaller than the MF pore size would pass through the system. It is possible that some of the aluminium floc particles would not be larger than the MF pore size, even after slow mixing and settling processes, and would pass through the MF membrane. Note that although a fraction of the bacteriophage population was not removed by the MF process, the removal ratio was very high. Enlargement of the floc size by

traditional treatment with coagulation and sedimentation processes is not necessary for the filtration with a ceramic MF (Lerch *et al.* 2005). In-line coagulation for 1 min was enough to meet the requirement for bacteriophage removal prior to the MF process.

Another possibility is that the contribution to virus removal by the cake layer or foulant may be the same in the three mixing types tested. Formation of the cake layer on the surface of the membrane and the deposition of foulant on the internal wall of the pore of the membrane over time plays an important role in the performance of virus removal not only during the direct MF process (Jacangelo *et al.* 1995; Madaeni *et al.* 1995) but also during the coagulation–MF process (Shirasaki *et al.* 2008). The amount of aluminium floc particles introduced into the MF module for both the in-line mixing system and the rapid mechanical mixing system was approximately three times as high as that for the mechanical mixing system followed by settling, because approximately 75% of the total amount of aluminium floc particles had settled before the water was fed into the MF module. However, the cake layer and foulant do not always contribute to virus removal (Shirasaki *et al.* 2008). It is possible that the extent of cake layer formation and foulant deposition contributing to virus removal is similar in all three types of mixing system.

Here, we propose that a PACl dose of 1.08 mg-Al/L and a coagulation time of 1 min are required to achieve high removal ratios of infectious and total viruses in the present in-line coagulation–ceramic MF system. Virus removal by the in-line coagulation–ceramic MF system is similar to that by the coagulation–ceramic MF system incorporating mechanical mixing. However, virus removal may be affected by the quality of the source water. To elucidate whether the coagulation conditions employed in the present study are sufficient for all water sources, further investigations using a wide variety of water sources are needed.

CONCLUSIONS

1. The performance of the in-line coagulation–ceramic MF system in removing the bacteriophages Qβ and MS was efficient at a coagulant dose of 1.08 mg-Al/L, and its

performance was similar to that of the mechanical coagulation–ceramic MF system. The in-line mixing system has potential as a useful pretreatment for viruses as an alternative to the mechanical mixing system for coagulation. Replacement of the mechanical mixing system with the in-line mixing system will reduce footprint, because there is no requirement for mixing/settling tanks for coagulation.

2. Coagulant dose and coagulation time were important factors controlling the virus removal performance of the in-line coagulation–ceramic MF system: a coagulant dose of 1.08 mg-Al/L and a coagulation time of 1 min were required to achieve high ratios of virus removal, >8.2-log for infectious and >5.4-log for total viruses, which satisfy the USEPA requirement of a 4-log removal/inactivation.

3. The removal performances of the coagulation–ceramic MF process for total Qβ and MS2 were different: the removal ratio for total MS2 was higher than that for total Qβ under all tested coagulation conditions, which was most likely the result of differences between Qβ and MS2 in their interaction with the cake layer.

ACKNOWLEDGEMENTS

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