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Effects of surface hydrophobicity on the removal of F-specific RNA phages from reclaimed water by coagulation and ceramic membrane microfiltration

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ABSTRACT

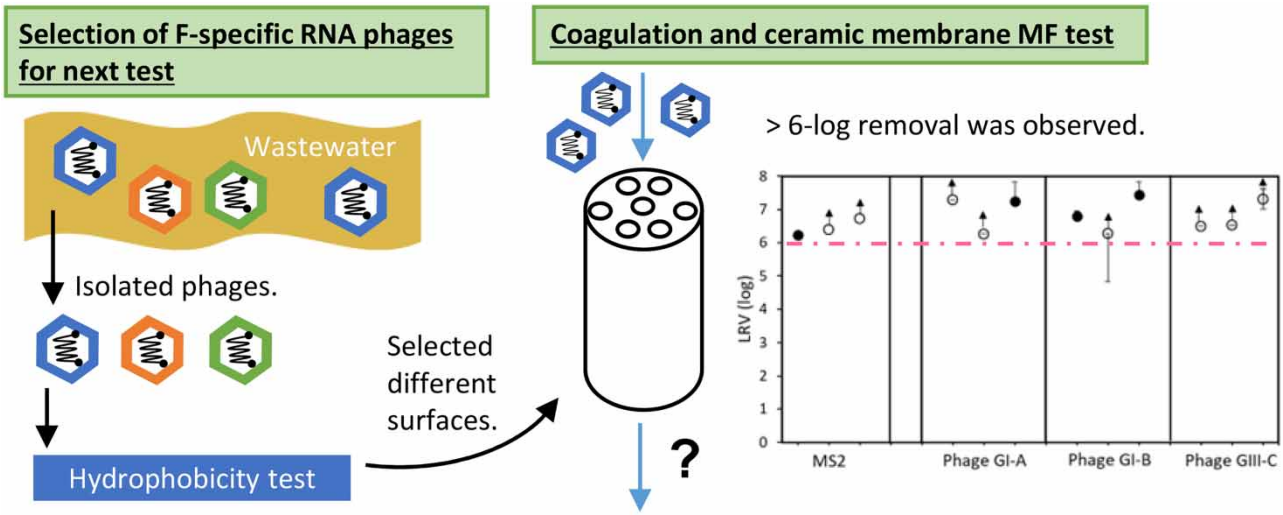
Microfiltration (MF) has been widely adopted as an advanced treatment process to reduce suspended solids and turbidity in treated wastewater effluents designated for potable reuse. Although microfilter pores are much larger than viruses, the addition of a coagulant upstream of a microfilter system can achieve stable virus removal. Ceramic membranes have a narrow pore size distribution to achieve the high removal of contaminants. This study aims to evaluate virus log reduction using bench-scale coagulation and ceramic membrane MF. To investigate the effects of differences in net surface hydrophobicity, 18 sewage-derived F-specific RNA phages (FRNAPHs) were used for batch hydrophobicity and coagulation–MF tests. The capability of bench-scale coagulation and ceramic membrane MF under continuous automated long-term operation was tested to remove the lab reference strain MS2 and three selected FRNAPH isolates which varied by surface property. Median virus log reduction values (LRVs) exceeding 6.2 were obtained for all three isolates and MS2. Although coagulation and hydrophobicity were positively correlated, the virus isolate demonstrating the lowest level of hydrophobicity and coagulation (genogroup I) still exhibited a high LRV. Thus, coagulation and ceramic membrane MF systems may serve as viable options for virus removal during water reclamation and advanced treatment.

Key words: ceramic membrane, coagulation, filtration, hydrophobicity, virus

HIGHLIGHTS

- Virus removal by a coagulation and ceramic membrane MF system was evaluated.
- The hydrophobicity of F-specific RNA phage isolates was investigated.
- A positive correlation was measured between hydrophobicity and coagulation of isolates.
- The least coagulated virus showed more than 6-log reduction by coagulation and ceramic membrane MF systems.

GRAPHICAL ABSTRACT



INTRODUCTION

Wastewater reuse has been in practice by human civilizations for several millennia (Angelakis & Gikas 2014). However, the advent of global climate change over the past century has led to temporal and spatial water scarcity issues that require more innovative and immediate solutions (Vo *et al.* 2014). Advanced water treatment technologies that are capable of removing microbiological contaminants of concern have been extensively researched including membrane-based processes. Direct and indirect potable water reuse systems have increasingly become viewed as implementable and practical options, especially in arid water-scarce areas. However, stringent guidelines, practices, and regulatory standards are required to properly manage and monitor treatment trains to ensure acceptable risk of pathogen infection.

Owing to their abundance and difficulty of removal during the process of water reclamation, human enteric viruses are considered as one of the most crucial targets for pathogen reduction. Up to 10^{12} human enteric viruses are excreted per gram of feces into wastewater by infected persons. However, only a few particles are required to cause infection and symptoms of illness (Kirby *et al.* 2014, 2015). Furthermore, enteric non-enveloped viruses are more resistant to environmental stressors and water disinfection compared to bacteria (WHO 2011). To guarantee accountability for the use of reclaimed water, sufficient virus removal is imperative to achieve an acceptable level of infection risk for end users.

The implementation of multiple barrier treatment processes is recommended to prevent pathogens and harmful contaminants from entering drinking water systems (Asano *et al.* 2007), thereby ensuring the microbial and chemical safety of drinking water (WHO 2011). In the state of California in the United States, a 12-$\log_{10}$ and 20-$\log_{10}$ reduction of viruses from raw sewage was set or recommended as a performance target for indirect and direct potable reuse (CCR 2015, 2021). In order to realize the reduction goals required by increasingly stringent water treatment regulations, several treatment processes that are capable of stable and consistent virus removal efficiencies are required.

Membrane treatment is a technology that has been employed in many water treatment facilities. However, the World Health Organization (WHO) reported validated log reduction values (LRVs) of viruses for water treatment processes (WHO 2017), and no reduction value was allocated to microfiltration (MF) since its virus removal efficiency is dependent on the treatment condition (Frohnert *et al.* 2015; Shirasaki *et al.* 2017a). However, when combined with a pre-coagulation step, MF exhibits higher levels of virus removal than with filtration alone (Zhu *et al.* 2005; Fiksdal & Leiknes 2006; Shirasaki *et al.* 2009; Guo & Hu 2011; Meyn *et al.* 2012; Lee *et al.* 2017). These studies indicated that viruses trapped within or adsorbed to flocs generated during the chemical coagulation process were retained by the membranes and thereby removed from the water stream.

The surface properties of viruses including net charge and hydrophobicity may vary even among closely related strains, thereby influencing their behavior in aqueous systems. During chemical coagulation, metal cations introduced into water destabilize colloids by neutralizing surface charge or by facilitating cationic bridging between negatively charged colloids

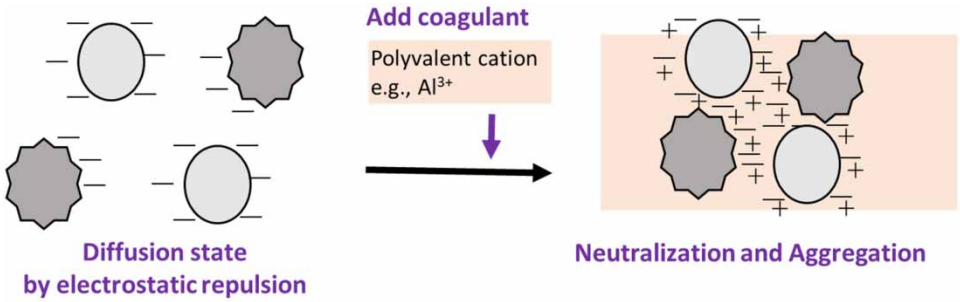


Figure 1 | Mechanism of chemical coagulation.

(Figure 1). Differences in chemical coagulation characteristics have been reported among viruses (Shirasaki *et al.* 2016). Shirasaki *et al.* (2016) reported that the magnitude of LRVs corresponded with increased virus hydrophobicity as the more hydrophobic viruses demonstrated greater levels of removal. Therefore, variation in net virus surface hydrophobicity may contribute to the observed differences in LRVs. The effects of virus hydrophobicity on LRVs achieved during the coagulation–MF treatment process warrant further research.

Pore size distribution is one of the important factors for improving virus removal by membranes. Water flow tends to pass through the membranes via larger pores (Jensen *et al.* 2014), and the broad distribution of larger pore sizes might result in lower virus removal efficiency. Urase *et al.* (1996) reported the presence of abnormally large pores in the UF membrane and surmised that these larger pores resulted in virus leakage from the UF membranes. Virus removal may therefore be more stable when the pore size distribution is uniform. Ceramic membrane microfilters are not only stable physically and chemically but typified by a narrow pore size distribution, which may contribute to greater and more consistent levels of virus removal when combined with a pre-coagulation step.

The objective of this study was to evaluate the LRVs of viruses by bench-scale coagulation and ceramic membrane MF systems under long-term automated operation using F-specific RNA phage (FRNAPH) laboratory strains and sewage-derived strains characterized by varying degrees of surface hydrophobicity. FRNAPHs are single-stranded RNA icosahedral phages with a diameter of approximately 26 nm (King *et al.* 2019), which is similar in size to enteric viruses. In an effort to account for the effects of morphological differences, 18 distinct FRNAPH isolates were initially used to investigate the effects of hydrophobicity on coagulation characteristics under buffered conditions. Three isolates were subsequently selected for further virus removal tests by coagulation–ceramic MF.

MATERIALS AND METHODS

Preparation of F-specific RNA phages

F-specific RNA reference bacteriophages MS2 (ATCC 15597-B1) and Qβ (ATCC 23631-B1) belonging to genotype I (GI) and III (GIII), respectively, and 18 FRNAPH environmental strains that had been isolated from raw sewage or from secondary treated wastewater (provided courtesy of Dr Haramoto, University of Yamanashi) were used in the study. Among the 18 environmental strains tested, 14 belonged to GI FRNAPHs and 4 to GIII FRNAPHs. Each FRNAPH was propagated overnight with the host bacteria *Escherichia coli* K12 A/λ F^+ in Luria Bertani (LB) broth solution. The FRNAPH propagation suspension was centrifuged (3,500 rpm, 5 min) and then filtered through a cellulose acetate filter (pore size 0.20 μm, Advantec, Tokyo, Japan). The concentration of each stock solution was determined by serial dilutions followed by the plaque assay to obtain approximately 10^{11} plaque-forming units per milliliter (pfu/ml).

Hydrophobicity testing of FRNA phages

The net hydrophobicity of MS2, Qβ, and each of the 18 wastewater FRNAPH isolates was evaluated by adsorption to hydrophobic beads (Brie *et al.* 2016). Beads coated with polystyrene (diameter: 1.5 μm, PolySciences, 19133) were used as the hydrophobic surfaces. Prior to use in the test, the beads were added to a phosphate buffer (pH 7.2) to obtain a target concentration of 1.35×10^6 beads/ml and rinsed twice in fresh volumes of the phosphate buffer by vortex mixing and centrifugation (10,000 g, 10 min). The binding assays were performed within a 15 ml Protein LoBind tube (Eppendorf, Sigma, USA) by seeding a 5 ml suspension of the phosphate buffer-bead suspension (pH 7.2) with test virus to achieve a concentration range of

10^4–10^6 pfu/ml. The inoculated hydrophobic beads were shaken at 150 rpm for 2 h at room temperature. All assessments were conducted in duplicate. Control tubes containing viruses in phosphate buffer solution only (no beads) were concurrently shaken under the same conditions as the binding assay tubes with beads. After the 2-h shaking period, the tubes with beads were centrifuged at 10,000 g for 10 min. The supernatant was clarified by the passage through a cellulose acetate filter (pore size 0.20 μm, Advantec). The bacteriophages in the binding assay supernatants and the control suspensions were then enumerated using the plaque assay method to assess relative levels of virus adsorption to the hydrophobic beads.

Batch coagulation and filtration tests

Aliquots of stock solution for each of the 18 sewage-derived FRNAPHs and the reference viruses MS2 and Qβ were added to 5 ml volumes of 0.1 M citrate Na-NaOH buffer (pH 5) at a 10,000-fold dilution to obtain approximately 10^7 pfu/ml. Polyaluminum chloride (High-basicity PACl, Hiei Co., Ltd, Japan) was then added at 50 mg/L and mixed by vortexing for 30 s. The solutions were then passed through cellulose acetate filters (syringe filter, pore size 0.20 μm, Advantec), and plaque assays were performed on the filtrates. To investigate the effects of the citrate Na-NaOH buffer (pH 5) alone, a filtration test without PACl was also conducted as a control using MS2 and Qβ. To evaluate the significant difference between LRVs, a one-way ANOVA test was conducted. Based on the batch MF with pre-coagulation test results, three indigenous FRNAPH isolates GI-A (LC710217, DNA Data Bank of Japan (DDBJ)) and GI-B (LC710218, DDBJ), which demonstrated the highest and lowest LRVs, respectively, and one GIII strain showing the highest LRV, described as GIII-C (LC710219, DDBJ), was selected for further coagulation and ceramic membrane MF experiments using reclaimed water.

Bench-scale coagulation and ceramic membrane MF tests

Spike tests were conducted in triplicate for each virus to test the removal ability of a ceramic MF membrane device (Metawater Co., Ltd, Japan) fitted with a pre-coagulation injection line at the Water & Energy Sustainable Technology (WEST) Center at The University of Arizona during December 2017, October 2018, and February 2019. The experimental process is shown in Figure 2. Reclaimed wastewater (also termed as tertiary effluent) sourced from the Agua Nueva Water Reclamation Facility co-located with the WEST Center (Tucson, AZ) served as the raw feedwater matrix used in the bench-scale coagulation–ceramic MF experiments. The reclaimed water is sand-filtered secondary effluent treated by chloramination delivered via a dedicated piping system from the Agua Nueva facility to the WEST Center. On the day prior to testing, 150 L bulk volumes of reclaimed water were pumped into a 100- or 200-gallon capacity tank and then held at room temperature overnight to facilitate dichlorination by evaporation. Free chlorine levels were verified prior to virus spiking using a Hach Pocket Colorimeter II (Hach Company, Loveland, CO, USA), and measurements $\leq$0.04 mg/L were considered as suitable testing to proceed. Table 1 shows the water quality parameters measured for the raw feedwater.

Aliquots of MS2 or FRNAPH stocks were spiked into the raw water feed volume (150 L) to obtain a target density range of 10^6–10^7 pfu/ml and mixed for 30 min. The raw water was then subjected to continuous treatment by pumping with inline injection of 50 mg/L PACl (PAX-XL 19, Kemira, USA) coagulant followed by the passage through an inline static mixer (1/2-N40-172-0, Noritake Co. Ltd., Japan) at a constant flow rate (500 ml/min). The coagulant-treated feedwater was then immediately filtered through the ceramic membrane MF (monolithic membrane, pore size 0.1 μm, membrane area

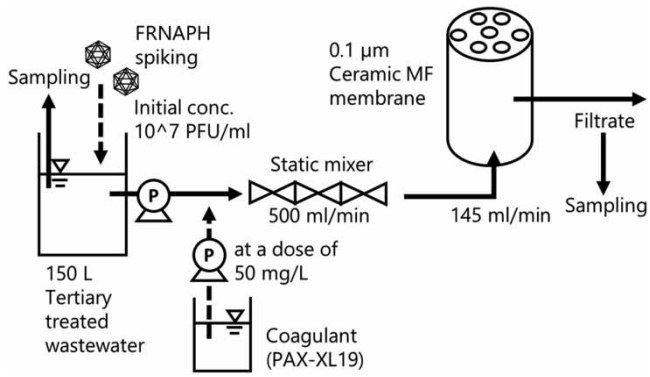


Figure 2 | Methodology of bench-scale coagulation and ceramic membrane MF system.

Table 1 | Water quality of tertiary-treated wastewater (raw feedwater for coagulation–ceramic MF experiments)

Water quality parameter (units)	Measured values
Turbidity (NTU)	0.8 ± 0.2
pH	7.1 ± 0.2
Conductivity (mS/m)	110 ± 10
TOC (mg/L)	7.0 ± 2.0

0.084 m^2, summarized in Table 2) at a flux of 2.5 m/day (=m^3/m^2/day) to simulate full-scale operational conditions at a water treatment plant. Filtrates were collected every 15 min with a total of four treated samples collected during the course of experimentation. Raw feedwater samples were collected concurrently with the filtrate samples to measure virus concentrations during experimentation. A backwash cycle (water and air for 1 min) was conducted following each spike assessment to clean and restore the condition of the membrane.

Virus quantification

Assessments of virus hydrophobicity and coagulation (batch tests), as well as the second bench-scale coagulation–ceramic membrane MF test, were conducted at the University of Tokyo. FRNAPH sewage isolates, MS2, and Qβ were measured at the University of Tokyo using the single-layer agar method with *E. coli* K12 A/λ F^+ as the host strain. Concentrations of viable FRNAPHs in the raw feed and filtrate samples were measured using the plaque-forming unit (pfu) assay technique. Sample aliquots were combined with log-phase host culture and LB agar, poured into Petri plates, and then incubated at 37°C overnight. Virus plaques were enumerated the following day and results were indicated in units of pfu per milliliter (pfu/ml).

The first and third bench-scale coagulation–ceramic membrane MF tests were conducted at the University of Arizona WEST Center. MS2 and sewage isolates of FRNAPHs were measured using the double-layer agar PFU assay method with *E. coli* 15597 at the host strain. Feedwater samples were serially diluted (1:10) in phosphate-buffered saline (pH 7.2), and aliquots of 0.1 mL were combined with 0.5 mL of log-phase host in glass tubes containing 5 mL of soft agar overlay (8 g/L agar–agar prepared in 30 g/L of tryptic soy broth) and briefly mixed by pulse vortexing. Treated filtrate volumes were assayed in aliquots of 2 mL at five tubes per sample to obtain the total assay volume of 10 mL; the sample volumes were then combined with log-phase *E. coli* 15597 and further processed as described. All feedwater and treated filtrate overlays were poured over the surface of tryptic soy agar plates and allowed to solidify at room temperature prior to inversion and incubation at 37°C overnight.

Real-time quantitative PCR (RT-qPCR) was also conducted at the University of Tokyo and the methods are described in the Supplementary Information.

RESULTS AND DISCUSSION

Hydrophobicity of FRNA phages

Hydrophobicity was evaluated by the adsorption test using the polystyrene beads as described previously for each environmental FRNAPH isolate, MS2 and Qβ. The LRVs measured in the liquid phase for each virus following the specified

Table 2 | Bench-scale ceramic membrane filtration setup

Membrane	Ceramic
Membrane type	Monolith
Pore size	0.1 μm
Membrane area	0.084 m^2
Flux	2.5 m/day (approximately 100 LMH)
Backwash length	1 min

period of adsorption to the beads indicated their hydrophobicity. The LRVs of the GI and GIII strains ranged from −0.037 to 2.7 and 1.9 to 4.1, respectively (Figure 3). The GIII strains including Qβ demonstrated higher levels of removal and therefore greater net hydrophobicity than most of the GI strains including MS2 (ANOVA, $p < 0.05$). The results described herein align with other published studies assessing relative levels of hydrophobicity for FRNA phages. Langlet *et al.* (2008) previously reported that Qβ was more hydrophobic than MS2 based on virus aggregation. Armanious *et al.* (2016) calculated the theoretical virus surface polarity based on the molecular capsid composition and revealed a higher net hydrophobicity for Qβ compared to MS2.

The average LRVs of MS2 and Qβ were 0.31 and 2.34, respectively, which were within the range of the LRVs measured for the GI and GIII isolates (Figure 3). Hydrophobicity varied moderately within the GI genotype isolates. However, one GI strain exhibited a much higher LRV at 2.7 that was more similar to those measured for the GIII strains. Overall, the FRNAPH isolates exhibited variable levels of hydrophobicity. The present work utilized isolates from wastewater in Yamanashi, Japan, which may be more diverse with regard to structural genes, thereby inducing differences in their hydrophobicity. Hartard *et al.* (2015) similarly detected various sequences of FRNAPH GI strains from wastewater.

Diversity of coagulation characteristics of FRNAPH laboratory strains and isolates during batch testing

In batch MF with pre-coagulation by PACl, LRVs of FRNAPH GI and GIII indigenous and laboratory strains ranged from 0.1 to 0.8 and >2.1, respectively (Figure 4). No removal was measured without PACl pre-coagulation (data not shown), indicating

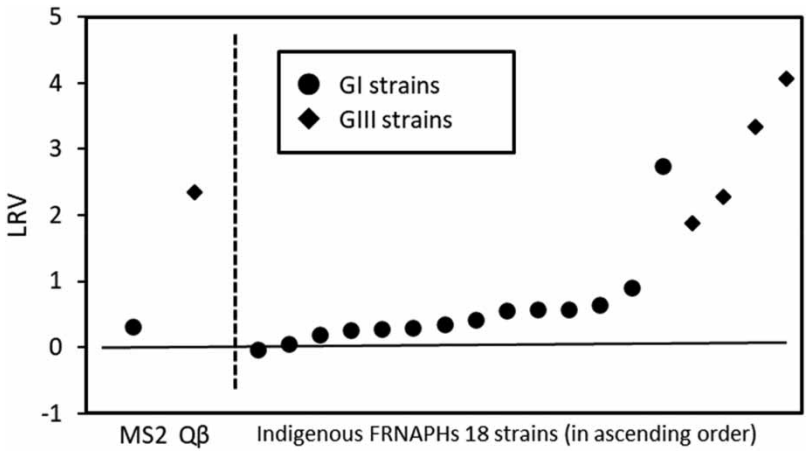


Figure 3 | LRVs of MS2, Qβ, and FRNAPH isolates by the adsorption test of hydrophobic beads. The average of two tests is shown as plots.

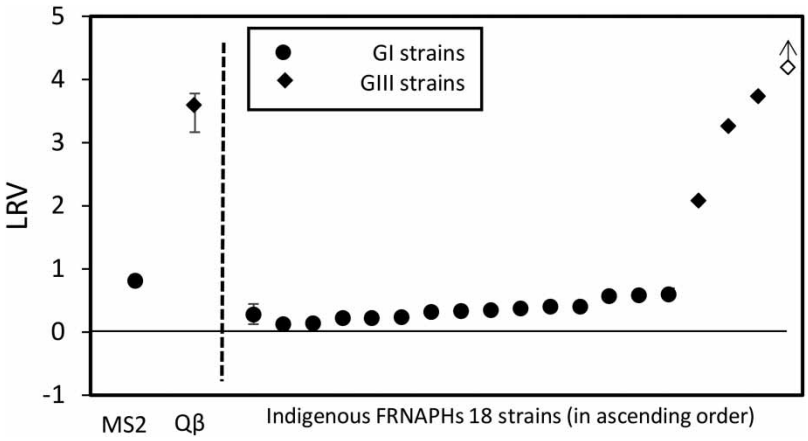


Figure 4 | LRVs of MS2, Qβ, and FRNAPH isolates by batch coagulation and filtration tests. MS2, Qβ, two plots (GI-A and GI-B) with an error bar: $n = 3$, others: $n = 1$.

that coagulation by PACl improved virus LRVs. The average LRV of MS2 was 0.82 ($n = 3$), exceeding that of the indigenous sewage-derived GI strains; Qβ also had a relatively high LRV of 3.6 compared with the indigenous GIII strains (Figure 4). This indicates that the widely used laboratory strains MS2 and Qβ do not fully represent the coagulation characteristics of the diverse environmental strains isolated from sewage. Some studies targeted indigenous FRNAPH as the marker of pathogen contamination in the environmental sample (Hartard *et al.* 2016) because these phages are excreted to feces and have host-specificity (e.g., FRNAPH GIII originates from humans) (Havelaar *et al.* 1990; Hartard *et al.* 2015). Although the laboratory strains have been used as model viruses instead of indigenous strains to develop the methods to concentrate indigenous strains, our results indicate that it may not be sufficient. Many virus concentration methods adopt the mechanism of virus adsorption to material by surface interactions (Ikner *et al.* 2012; Shi *et al.* 2017); therefore, the surface diversity of isolates may affect recovery rates. And, of course, human enteric viruses probably have a variation in surface properties. The review by Michen & Graule (2010) reported several isoelectric points (pIs) for one virus. However, for most of them, pIs are not varied dramatically. For example, reported pIs of human echovirus 1 were 4.0–6.4, indicating that this virus has a negative charge in neutral conditions and may not show a clear difference during water treatments unless conducted under acidic conditions such as pH 5. Hydrophobicity diversity may be more important than charge diversity except for certain water qualities.

LRVs of the four GIII strains significantly exceeded those of the 14 GI strains (ANOVA, $p < 0.05$), suggesting that the coagulation characteristics of FRNAPHs vary between genotypes. Almost all GI strains were less hydrophobic than the GIII strains (Figure 4), thereby supporting the contribution of virus hydrophobicity as a factor in coagulation as mentioned in Shirasaki *et al.* (2016). During the inline chemical coagulation process, metal cations such as Al^{3+} (a component of PACl) neutralize the net negative surface charge of viruses and other particles, thereby facilitating their aggregation and adsorption onto flocs. In our results, floc formulation appears to be enhanced by increasing levels of particle hydrophobicity. The correlation between hydrophobicity and coagulation characteristics is evaluated in the next section.

Interestingly, the LRVs of GI strains ranged from 0.12 to 0.69 even within the same genotype (Figure 4). A significant difference between the highest (GI-A, average 0.59, $n = 3$) and the lowest LRVs (GI-B, average 0.27, $n = 3$) was observed within GI strains (ANOVA, $n = 3$, $p < 0.05$). Hereafter, the LRVs for the pre-coagulation and filtration tests of each isolate were assumed as more comprehensive parameters to represent their coagulation characteristics and used in further discussion.

Relationship between coagulation characteristics and hydrophobicity

A positive correlation was measured between coagulation and hydrophobicity of FRNAPHs ($R^2 = 0.67$, Figure 5), implying that the higher net hydrophobicity of the GIII strains contributed to their greater coagulation capacity in the batch test. This further indicates that the hydrophobicity of viruses is a contributing factor in their tendency to coagulate. To demonstrate the sufficient removal of viruses by coagulation, LRVs should be evaluated using viruses characterized by high, moderate, and low levels of surface hydrophobicity.

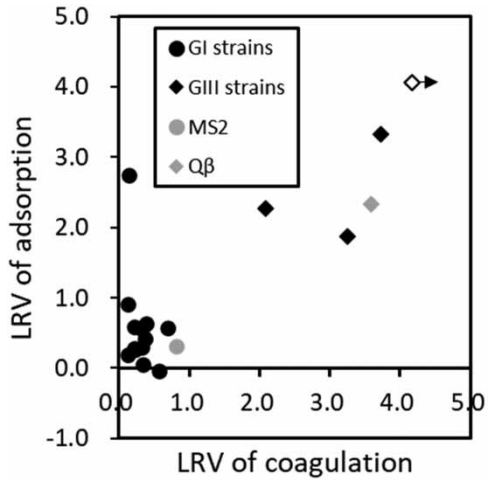


Figure 5 | LRVs of MS2, Qβ, and FRNAPH isolates by the coagulation test and by the adsorption test. The open plot shows the detection limit.

In addition to hydrophobicity, other factors such as the net surface charge can affect the ability of viruses to coagulate. Among the GI strains, no clear relationship was observed between coagulation characteristics and hydrophobicity (Figure 5). One strain showed high hydrophobicity similar to the GIII strains, but its coagulation characteristic did not differ from the other GI strains. A significant difference in coagulation characteristics within the genotype was also observed in the batch coagulation test. Our results indicated the risk by use of a small number of viruses for the removal test by coagulation and filtration. Usually, the challenge test is conducted with laboratory strains, meaning surface diversity in the environmental samples is not considered. Virus removal rates in the actual treatment plants can be affected by variations of the strain, especially those hardly removed. For example, the difference in chlorine resistance among the same genotype is also reported in previous studies (Meister *et al.* 2018; Torii *et al.* 2021). Although isolate GI-A was less hydrophobic than isolate GI-B, the LRV of GI-A (average 0.59, $n = 3$) significantly exceeded that of GI-B (average 0.27, $n = 3$) (ANOVA, $p < 0.05$). This may be attributed to the capsid electrostatic charge that is governed by ionized amino acids (Penrod *et al.* 1996). Mayer *et al.* (2008) reported that viruses that have relatively high pIs (relatively positive charge) showed the higher LRVs by coagulation, indicating that electrostatic charges are also important to determine virus removal by coagulation. Further studies are needed to investigate the relationship between virus surface charge and coagulation characteristics.

Bench-scale coagulation and ceramic membrane MF experiment

During the bench-scale coagulation and ceramic membrane MF experiment, raw water and filtrate were collected four times every 15 min during one continuous filtration period. No time dependence was found in virus feed concentration quantified by either the plaque assay or RT-qPCR (data not shown); therefore, the average virus concentrations were used as virus feed concentrations. For the following discussion, the plaque assay results are referenced rather than RT-qPCR-based results owing to the smaller standard deviation of the culture infectivity method (GI-A: 0.33, GI-B: 0.23) compared to the molecular assay (GI-A: 0.74, GI-B: 0.46) (Supplementary Tables S1 and S2). The observed differences between the methods may be attributed to qPCR-based quantification of free RNAs and RNA fractions present in the stock solution used for spiking of the raw feed, which may be less easily removed during the coagulation and filtration process; therefore, the LRVs based on the qPCR were lower with more variability than those yielded by the plaque assay (Supplementary Table S1). Considering that the removal of encapsidated viruses is more relevant for assessing LRVs of infectious particles than free RNA, and that qPCR quantification does not differentiate RNA whether free or capsid-associated, discussions hereafter are based on the plaque assay results.

In the coagulation and ceramic membrane MF experiment, sewage-derived FRNAPH isolates GI-A, GI-B, and GIII-C as well as MS2 exhibited LRVs exceeding 6.2 based on the plaque assay (Figure 6) with the initial virus concentrations ranging from 6.3 to 7.8 log pfu/ml and the detection limit at 1 pfu/ml. GIII-C, which showed the highest coagulation characteristics and the highest hydrophobicity in the batch test, was below the detection limit for most of the filtrate samples during the bench-scale coagulation and ceramic membrane MF experiment. Interestingly, the GI strains (GI-A, GI-B) also exhibited

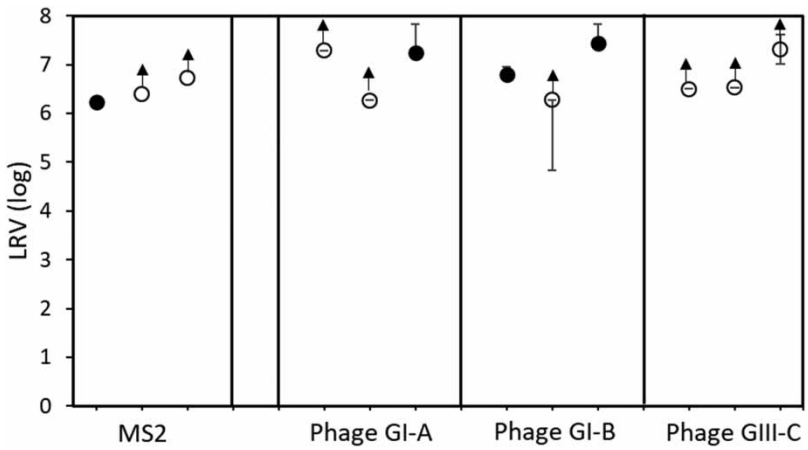


Figure 6 | LRVs by the inline coagulation and MF system. The MS2 plots are the results of only one sample. The plots of phage GI-A, GI-B, and GIII-C show a median of four samples during 1 run. If an undetected virus sample is included, it is displayed as open plots. Error bars indicated the data range.

comparable LRVs to the GIII strain, which exceeded 6.2, showing that there was no significant difference in removal between the genotypes.

A gap in the LRV between the results of the batch test and the bench-scale coagulation and ceramic membrane MF test was observed, with the latter exhibiting much higher LRVs (>6.2) than the former (0.27–2.4). Several parameters likely contributed to the differences observed between the outcomes of the two tests – the most critical one being water quality (buffer vs. wastewater). The results indicate that coagulation was enhanced by the turbidity and organic composition in the reclaimed water matrix used in the coagulation and ceramic membrane MF experiment compared to the batch test that used citrate Na-NaOH buffer (pH 5.0). Lee *et al.* (2017) reported the difference in LRVs according to water quality (e.g., organic matter and pH) by coagulation and filtration. Interestingly, even within the wastewater, virus LRVs decreased with increasing organic matter concentration. The results of Meyn *et al.* (2012) also indicated that high natural organic matter concentration increased the necessary coagulant dose. In terms of the pH of the feedwater, Ding *et al.* (2016) reported that a relatively acidic pH value was required to neutralize micro particles' surface charges in environmental water, indicating that the necessary coagulant dose can decrease in acidic pH. Actually, viruses were removed a lot in pH 5.5 as described by Lee *et al.* (2017). These parameters can be a critical factor in determining virus LRVs by coagulation and filtration. The challenge test to find suitable coagulation conditions should be done prior to employing the coagulation and ceramic membrane system.

Membrane types also differed between the two tests; the ceramic membrane's pore size (0.1 μm) was smaller than that of the batch test's membrane (0.2 μm) and its pore size distribution was sharp. The results of Urase *et al.* (1996) realized the risk of virus leakage from abnormally large pores. Our results may indicate the importance of pore size distribution. The favorable features of the ceramic membrane may also be the factors in obtaining high virus LRVs.

The coagulant's higher basicity in the ceramic MF test compared to that used in the batch test may also promote virus removal and affirms the findings by Shirasaki *et al.* (2017b) that high-basicity PACl is an effective coagulant for the removal of viruses.

The water treatment system employing PACl coagulation followed by the ceramic membrane MF system achieved the stable removal of FRNAPH viruses (LRV >4.8), and the median values of each virus LRV exceeded 6.2 (Figure 6). According to the State Water Control Board of California (2015), no single water treatment is to be credited with more than 6-$\log_{10}$ reduction (CCR 2015). Given the LRVs of greater than 6-$\log_{10}$ demonstrated herein for reclaimed water spiked with sewage-derived FRNAPH viruses and MS2 characterized by varying levels of net hydrophobicity, pre-coagulation with high-basicity chemistry like PACl followed by the ceramic membrane MF system represents a promising water treatment option for virus removal. More research is needed to assess the effects of water quality on the process of coagulation and floc formation. A more thorough, rigorous evaluation is warranted to formally assign log removal credits for this system as an advanced water treatment process for potable reuse.

CONCLUSIONS

This study investigated the effects of differences in net surface hydrophobicity using 18 sewage-derived FRNAPHs and evaluated the log reduction of MS2 and selected FRNAPHs using bench-scale coagulation and ceramic membrane MF. The net surface hydrophobicity positively correlated with coagulation in batch tests ($R^2 = 0.67$). Interestingly, laboratory strains (MS2 and Qβ) did not have the average hydrophobicity among the isolates, and hydrophobicity and coagulation characteristics were significantly different between GI and GIII strains. Some strains also showed a significant difference within the same genotype. Based on batch hydrophobicity and coagulation and filtration tests, three sewage-derived FRNAPHs which varied by surface property were selected for ceramic membrane tests under continuous automated operation. Median virus LRVs exceeding 6.2 were obtained for all target viruses including the virus isolate with the lowest level of hydrophobicity and coagulation, which belongs to FRNAPH GI. Although the effect of the feedwater quality should be investigated carefully, the coagulation and ceramic membrane MF system may be a promising option for virus removal during water reclamation and advanced treatment.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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