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N. Jacquet [a,b], S. Wurtzer [a], G. Darracq [a], Y. Wyart [b], L. Moulin [a], P. Moulin [b,*]

[a] Eau de Paris, Direction de la Recherche du Développement et de la Qualité de l'Eau, 33 Avenue Jean Jaurès, 94200, Ivry-Sur-Seine, France
[b] Aix Marseille Univ., CNRS, Centrale Marseille, M2P2 UMR 7340, Equipe Procédés Membranaires (EPM), Europôle de l'Arbois, BP80, Pavillon Laennec, Hall C, 13545, Aix en Provence Cedex, France

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ABSTRACT

Removal of pathogenic microorganisms as viruses during drinking water production was evaluated by ultrafiltration. Two enteric viruses (ADV 41 and CV-B5) were compared to the MS2 bacteriophage, largely used in literature and by membrane producers as enteric virus surrogate. The effect of feed concentration of viruses on the ultrafiltration efficiency has been assessed. For the three viruses, low retentions about 1 log were observed at the lowest concentrations. At higher concentrations, an increase of removal up to 3.0 log for CV-B5 and MS2 phage and 3.5 log for ADV 41 was observed. These results highlight the potential overestimation of UF efficiency during laboratory experiments realized at high concentrations, compared to low concentrations found in environmental resources used for drinking water production. Virus removals with Evian water and real groundwater were compared and groundwater achieved similar or slightly higher removals for the three viruses. Finally, impact of membrane ageing after chlorine exposure was checked. It was observed that membrane degradations, visible by a water permeability increase with exposure dose did not affect the removal of viruses at low feed concentrations.

1. Introduction

In drinking water production, ensuring a pathogenic microorganisms free water is a major concern because of the sanitary risk. The microbiological water quality is guaranteed by the monitoring of fecal indicator bacteria. Enteric viruses are not included in the water regulatory control, but the annual reports of the European Food Safety Agency highlight the predominant role of enteric viruses in the reported waterborne acute gastroenteritis. The 4th candidate contaminant list published by USEPA (CCL 4) contains some of these pathogens potentially present in water: adenovirus (ADV), calcivirus, enterovirus (EV) and hepatitis A virus (HAV) [1]. These viruses can result in various affections as respiratory and/or gastrointestinal illness for adenovirus, or liver disease and icterus for HAV for example. Enteroviruses can also cause a large spectrum of human illness from mild febrile illness to more severe forms (neo-natal multi organ failure, paralyzing poliomyelitis, myocarditis etc.) [2,3]. Adenoviruses, noroviruses and enteroviruses are some of the major viruses reported in surface and groundwater resources [2,4,5]. Drinking water treatment plants (DWTP) ensure a multi barrier treatment against these pathogens, including viruses. Such treatment could include Ozonation, UV radiation, monochloramine (outside Europe) or free-chlorine disinfections, but all viruses are not similarly removed by each method and can be resistant to treatments. For example, ADV are reported to be highly resistant to UV disinfection [2,6]. Coxsackievirus-B5 (CV–B5) is also more resistant to free chlorine than other serotypes of enteroviruses [6]. In addition to these disinfection treatments, physico-chemical treatment, and especially ultrafiltration (UF) is reported as a potential virus removal treatment. UF is more and more used in DWTP and can be considered to replace or complete some pretreatments without chemical addition [7,8]. UF efficiency concerning microbial removal has already been well documented [3,7,9,10]. More than 5 log removal for *Giardia* and *Cryptosporidium* [9], and 4 log removal for *E. Coli* were observed [7]. Some studies also focused on viral removal by UF, with different viruses, and reported different removal efficacies from 3 log to more than 5 log (ADV, EV, HAV etc.) [6,7,11]. Bacteriophages are often used as virus surrogates due to their fast and easy culture and their innocuity for humans. UF retention efficiency has been largely studied regarding MS2 phages [9–20], Qβ phages [17], ΦX174 phages [16,18,20] and PP7 phages [21]. However, different results between studies can be observed and could be explained by

* Corresponding author.
E-mail address: philippe.moulin@univ-amu.fr (P. Moulin).

protocol differences, which make global comparisons and conclusions difficult. Viral retention is dependent on numerous factors as membrane Molecular Weight Cut-Off (MWCO) [9], membrane material [10,12,16], membrane fouling [11] and Transmembrane Pressure (TMP) applied during filtration [18] for example. Another factor that could influence retention efficiency is the viral concentration in solution. In studies, virus concentrations can differ and are often chosen with regards to analysis detection limits and simplicity of protocol but not from real environmental data. These studies used mostly artificially high virus concentrations compared to those found in raw groundwaters. World Health Organization (WHO) reports viral concentrations of enteroviruses and rotaviruses in raw water from 10^{-2} to 10^{2} viruses per liter [2], when assay are usually done using at least 10^{6} viruses per liter [8,9,11–13,18–20,22–25]. Jacangelo et al. have studied the influence of feed concentration on retention and observed a stable retention of MS2 phages on UF membranes from 10^{5} pfu.L^{-1} to 10^{9} pfu.L^{-1}, and then a retention decrease over 10^{9} pfu.L^{-1} [9]. Study of lower concentrations in the range of raw water used for DW production needs to be assessed to evaluate impact on viral retention and check the relevance of studies with high concentrations in viruses. Moreover, matrix effects, such as feed solution turbidity [25], pH and ionic strength [8] can modify hydrophobic and electrostatic virus-membranes [8,20] and virus-virus [26] interactions and affect filtration efficiency. For instance, when pH is close to the isoelectric point of the studied virus, aggregation phenomena are promoted, resulting in larger apparent sizes and then the retention mechanisms may be impacted [27]. The potential viral removal depends on the studied virus [17,28] and the feed water quality [8,9]. Thus, high quality feed solutions as demineralized water are mostly used in laboratory studies but it could give rise to different results in comparison with raw water in DWTP. Various studies showed contradictory results about matrix effects of solution on virus removal. Ferrer et al. concluded on no influence of the feed water quality on retention performance [7], while other authors highlighted the impact of water composition and quality on UF efficiency [9–11,25]. Finally, impact of membrane ageing due to chlorine cleanings on viral retention efficiency was assessed. Chlorine exposure effects have been largely studied with regards to membrane degradation, structure and filtrations characteristics. Pore size increase [29–32], change of surface hydrophobicity [29,33,34] and/or rugosity [29,35] for example, have been explained by degradation of membrane materials after chlorination. Most of the degradations have been attributed on membrane additives, such as PVP [36–38]. Robinson and Bérubé have studied membrane ageing in full scale DWTP and highlighted that permeability measurements are good indicators of membrane ageing [39]. Retention evolution with membrane ageing has already been assessed according to various compounds. Studies about organic compounds [37], *E.coli* and total bacteria [33,40], and bovine serum albumin (BSA) [30,34,35] retentions after chlorine exposure have given different results. Moreover, Ravereau et al. observed MS2 retention and showed an increasing retention on aged membranes [40], when other studies at industrial scale observed a loss of 1 log in MS2 phage retention for 6 years-old membranes [19,25].

The objectives of this study were to assess impacts of various experimental conditions on UF efficiency towards viral retention. In this study, impacts of viral concentration on removal of viruses were assessed on native membranes regarding sanitary risk. Concerning the impact of water composition, Evian (lab-used water) and raw water (industrial conditions) were compared to verify if the results with Evian in laboratory can be consistent with industrial evaluation. Then, membrane ageing after chlorine exposure was characterized in terms of water permeability and virus retention. For this study, various viruses were studied regarding the viral sanitary risk in DW and a virus model surrogate efficiency. Virus removal efficiency was estimated for adenovirus type 41 (ADV 41) and coxsackievirus-B5 (CV–B5). The virus surrogate used in this study was MS2 bacteriophage, largely used in the literature.

2. Material et methods

2.1. Membranes

Membranes used were polymeric multichannel hollow fibers (ALTEON™ I, SUEZ Aquasource®, France), built with hydrophilic polyether sulfone (PES) and a porogenic hydrophilic polymer (polyvinylpyrrolidone, PVP). One fiber was composed of 7 channels with an inner diameter of 0.9 mm. The external fiber's diameter was 4 mm. The active length of fiber was 20 cm which provided a specific surface of 3.96 10^{-3} m^{2} and an internal volume of 0.89 mL. The average initial membrane permeability with ultrapure water was 900 ± 100 L h^{-1} m^{-2}. bar^{-1}. The membrane pore size distribution was centered about 20 nm with a corresponding molecular weight cut-off (MWCO) between 150 and 200 kDa. This membrane is used to produce drinking water in France (Nancy, Orléans, l'Hay les Roses), Croatia (Dubrovnik), Switzerland (Lutry), Italia (Castiglione de Fiorantino), etc.

2.2. Filtration

Membrane modules were made of one multichannel membrane into a PTFE external shell with an epoxy plug on one side of the module, allowing in/out frontal filtration. After the module potting, membranes were rinsed with ultrapure water (300 L.m^{-2}) under a varying TMP with a maximum at 1.0 ± 0.1 bar to remove the preservative agent (glycerin) [41]. Water permeability was then measured with ultrapure water. Experiments were performed on vertical dead-end filtration mode. The feed suspension was pumped thanks to a peristaltic pump (Masterflex L/S number 7523–80) and went through the membrane at a constant flow 100 L.h^{-1}.m^{-2}. The TMP, temperature and permeate flux were continuously monitored during the experiments. Dead-end filtrations were processed up to a Volume Concentration Factor (VCF) of 450, which was near the range of the industrial VCF in drinking water production plant. The VCF (Eq. (1)) was calculated using the volume of permeate (Vp in mL) and the volume of retentate (Vr in mL).

$$VCF = \frac{Vp + Vr}{Vr} \quad (1)$$

Membranes were single used to avoid any contamination between experiments. Indeed, virus fouling is not totally irreversible. A new membrane module was used for each experiment. The feed solution was a suspension of viruses in Evian water (mineral water). Different experiments with varying concentration in feed were compared to evaluate the impact of initial viral concentration on the filtration efficiency. For each experiment, the concentration of virus in the feed and permeate were evaluated by RT-qPCR. The concentration range of viruses in feed varied from 10^{2} to 10^{10} viruses.L^{-1}.

2.3. Virus

Three different viruses were used in this study: ADV 41, CV-B5 and MS2 phage. The main characteristics of these viruses are presented in Table 1. All viruses present isoelectric point (pI) in the same range, inferior to the value of neutral pH and are therefore negatively charged in the experimental conditions of this study. They also present a similar shape with an icosahedral capsid. It is noticeable that CV-B5 and MS2 phage have very similar characteristics: they are both single stranded RNA viruses in the same size range (30 and 27 nm respectively) with similar molecular weight (MW), while ADV41 is a double stranded DNA virus with a size approximately three times larger than the two others (90 nm) and it also presents a larger MWCO.

ADV 41, CV-B5 and MS2 phages were cultivated on monolayer cultures of 293 A cells, Buffalo green monkey kidney (BGMK) cells and *E. coli* cells, respectively. Cultures were done at 37 °C with 5% CO_2. After visualization of cytopathic effects, supernatants of eukaryotic cell cultures and bacteria culture were mixed with chloroform (10% v/v) and

Table 1
ADV41, CV-B5 and MS2 phage characteristics.

	Size (nm)	pI (isoelectric point)	DNA/RNA virus	MW [6] (x10^3 kDa)	Shape
ADV 41	90	2-6 [42]	Double stranded DNA	150–180	Icosahedral capsid, non-enveloped virus
CV-B5	30	3.6 [43]	Single stranded RNA	8–9	
MS2 phage	27	3.5–3.9 [12,20]	Single stranded RNA	4	

clarified by centrifugation 8 000 xg for 15 min at 4 °C. Virus suspensions were concentrated up to 1.0 mL using UF membranes 30 kDa concentrators (Vivaspin 20, 30 kDa, Sartorius). Virus suspensions were purified on sucrose 40% cushion by ultracentrifugation at 150 000 g for 2 h at 4 °C. Virus concentrates were resuspended, and sucrose was finally diluted by two successive washes with phosphate-buffered saline (PBS) and concentration on UF devices (Vivaspin 20, 10 kDa, Sartorius). Viral stocks were quantified by RT-qPCR and stored at −80 °C until use. Dilutions of the viral stock solution were done in Evian water to obtain the initial amount of virus necessary for the filtration. Prior to filtration, viruses were treated with Ethidium monoazide (EMA) to inhibit the analysis by RT-qPCR of free nucleic acids. Protocol of EMA treatments was described elsewhere [5]. Briefly, EMA was added to the virus concentrate at a final concentration of 100 µM. Covalent binding of EMA with accessible genomes (free or degraded capsid) in sample inhibits their amplification by RT-qPCR. Thus, analyses of the experiments were focused only on virus having a genome protected inside a non-permeable capsid. This EMA treatment allowed to overcome the inconvenient of RT-qPCR analysis which does not consider of virus infectivity [5,44]. 200 mL of feed suspension were sampled for analysis. After filtration, the feed 200 mL sample and the global volume of permeate resulting from filtration were both concentrated on UF devices (Vivaspin 20, 10 kDa, Sartorius). Virus concentrates were directly lysed on the membrane by addition of a lysis buffer (ATL buffer, QIAGEN, 19076) up to a total volume of 1.0 mL. For experiments conducted with high viral concentrations, 200 µL of feed and permeate samples were directly extracted without concentration on vivaspin devices and ATL buffer addition. Viral nucleic acids from feed and permeate samples were extracted with a MagNA Pure compact system (Roche Applied Science, Bâle, Switzerland) and MagNA Pure Compact Acid Isolation Kit - Large volume, which allow processing the all sample. Nucleic acids were eluted into a 50 µL final extract [5]. Extracts were purified on PCR Inhibitor Removal Kit (Zymo Research Corporation, Irvine, CA) to remove any trace of free EMA residual able to inhibit amplification and immediately analyzed by RT-qPCR. Feed and permeate analyses allowed to assess the viral retention efficiency of the filtration. Each reaction was carried out with 10 µL of nucleic acid samples, 5 µL of master mix TaqMan® Fast Virus1-Step (Life Technologies, Carlsbad, CA), and 5 µL of mix containing primers and probes. Specific primers and probes designed for bacteriophage MS2, coxsackievirus B5 (Enterovirus) and adenovirus 41 (Adenovirus F) are presented in Table 2 with final concentrations in mix. The thermal cycling profile is described elsewhere [45]. Each RT-qPCR assay included a positive and a negative amplification control.

2.4. Log removal value (LRV) calculation

RT-qPCR analysis allowed to quantify the concentration of viruses contained in feed and permeate samples and evaluate the virus retention efficiency by calculating the LRV, depending on C_f and C_p (in viruses. L^{-1}), the concentrations of viruses in feed and permeate respectively (Eq. (2)).

$$LRV = log_{10}(\frac{Cf}{C_p}) \quad (2)$$

The quantification limit of RT-qPCR analysis was 15 viruses/reaction. Because only 10 µL out of the total 50 µL eluted volume was used for analysis, the quantification limit for the total sample was 75 viruses.

2.5. Comparison of Evian and groundwater

Raw groundwater was supplied from a DWTP (Eau de Paris, Saint-Cloud DWTP, France). The compositions of groundwater and Evian water were described in Table 3. Raw water was doped with viruses at low concentrations (<10^4 viruses.L^{-1}). The viral suspension was treated with powder activated carbon (PAC) 1.0 mg L^{-1} with a contact time of 30 min before UF process to replicate the industrial conditions from the DWTP. Feed samples were collected for analysis before and after PAC treatment. The LRV_{PAC}, depending on C_{f1} and C_{f2}, the viruses' concentrations before and after activated carbon treatment respectively (Eq. (3)), was calculated as 0.4 ± 0.1, 0.1 ± 0.1, 0.3 ± 0.2 respectively for ADV41, CV-B5 and MS2 phages. The UF LRV as defined in equation (2) is calculated depending on C_{f2} and C_p, the viral concentration in permeate (in viruses.L^{-1}).

$$LRV_{PAC} = log_{10}(\frac{C_{f1}}{C_{f2}}) \quad (3)$$

2.6. Membrane ageing

2.6.1. Ageing protocol

Membranes were passively exposed to chlorine by immersion into a NaOCl solution, provided from an industrial DWTP (Eau de Paris, Saint-Cloud DWTP, France). This NaOCl solution was industrially used as membrane chemical cleaning agent. Before membrane exposure to chlorine, elimination of preservative agent on the membrane surface was achieved by soaking of the fiber in ultrapure water for 72 h. The soaking bath was refreshed every hour during the first 5 h, and then every 24 h. This deconditioning step replaced the membrane deconditioning by ultrapure water flushing, achieved for non-aged membranes

Table 2
RT-qPCR mix composition [5].

Virus	Oligonucleotide	Sequence	Final concentration (nM)	Target
Bacteriophage MS2	MS2_F632	GTCGCGGTAATTGGCGC	100	maturation protein
	MS2_R708	GGCCACGTGTTTTGATCGA	300	
	MS2_P650	FAM-AGGCGCTCCGCTACCTTGCCCT-BHQ1	300	
Enterovirus	EV_F453	GCCCCTGAATGCG	900	5′-UTR
	EV_R583	TGTCACCATAAGCAGY	900	
	EV_P536	FAM-CCAAAGTAGTCGGTTCC-NFQ MGB	100	
Adenovirus F	ADV_F_F102	CACCGATACGTACTTCAG	600	hexon
	ADV_F_R231	GCGCACTTTGTAAGARTA	900	
	ADV_F_Ps160	Yakima Yellow-CACGATGTAACCACAGACAGG-BHQ1	200	

Table 3
St Cloud and Evian water composition.

	pH	Conductivity (µS.cm^{-1})	Ionic strength (mM)	TOC (mg.L^{-1})	Anions (Cl^-, NO_3^-, SO_4^{2-}) (mg.L^{-1})	Cations (Ca^{2+}, Mg^{2+},K^+,Na^+) (mg.L^{-1})	Alkali strength (°F)	Degree of hardness (mg$CaCO_3$.L^{-1})	Turbidity (FNU)	UV_{254} (cm^{-1})
Evian water	7.2	590	10	< 0.20	27.8 (10, 3.8,14)	113.5 (80, 26, 1, 6.5)	29.5	300	–	–
Groundwater	8.0	590	10	0.64	84.4 (24.4, 44, 16)	116.1 (97.3, 5.5, 2.3, 11)	21.8	275	0.30	0.015

[41]. This adapted protocol was due to the not potted membranes before ageing, not allowing flushing. Membranes were aged using a 30 mgCl_2.L^{-1} free chlorine solution at a regulated temperature of 25 °C. The pH of ageing solution was adjusted at 7.5 with sulfuric acid (H_2SO_4). The free chlorine concentration [46–48] and pH [49] of the solution are two major factors influencing the membrane degradations. These conditions have been chosen to be as close as possible to the industrial conditions. The chlorine solution was regularly renewed to maintain the chlorine concentration. Ageing was performed until various total exposure doses up to 45 000 mgCl_2.h.L^{-1} free chlorine. After the ageing process, fibers were rinsed by immersion in a solution of sodium thiosulfate 0.1 M ($Na_2S_2O_3$) to ensure end of potential reaction of chlorine on membrane material. The absence of membrane deterioration by a sodium thiosulfate exposure has been checked by immersion for 4 days. The water permeability of sodium thiosulfate exposed membranes was checked and was not affected, compared to native membranes (data not shown). After chlorine exposure, water permeability was measured, and virus filtration experiments were achieved. Membranes water permeability and virus retention were compared with data obtained with native membranes experiments.

2.6.2. *Filtrations protocol*

Aged membranes were tested with different levels of viral feed concentrations, designated as “high”, “intermediate” and/or “low” concentrations for each virus. Data in Table 4 detail the range of concentrations in viruses.L^{-1} of each level. These levels were defined after the experiments and the retention obtained with native membrane.

2.6.3. *Data analysis*

For retention evolution with membrane ageing, comparison between LRV of native and aged membranes has been assessed using Kruskal-Wallis test and Dunn’s multiple comparison test, assuming a non-Gaussian distribution of residuals. Significance level p of 0.05 was set for the analysis. For the comparison of LRV of CV-B5 when feed concentrations were intermediate and low, because only pairs of values were compared, a Mann-Whitney test was assessed with the same significance level p of 0.05.

3. Results and discussions

3.1. *Effect of feed viral concentration*

Impact of feed concentration (Cf) on the retention of different viruses was assessed (Fig. 1). It must be noted that viruses were detected and quantified in all the feed and permeate samples allowing to estimate a LRV in each experimental condition. For the three studied viruses, results showed an increase in virus removal depending on feed concentration. ADV 41 and CV-B5 removal evolutions showed similar tendencies. The lowest feed concentrations used (below 2.10^3 viruses.L^{-1}) showed less than 1.0 log removal for both viruses. An increase of LRV from feed concentration from 10^3 to 10^{5-6} viruses.L^{-1} was observed. In this concentration range, the leak of viruses in permeate was constant (10^2 to 10^3 viruses.L^{-1}), and the increase of LRV was caused by the feed concentration increase. For concentrations higher than 10^6 viruses.L^{-1}, the increase of permeate concentration led to the stabilization of ADV 41 and CV-B5 LRV at 3.5 and 3.0 log respectively. For MS2 phages retention, LRV was stable (under 1.0 log) for feed concentrations under 10^6 viruses.L^{-1}. Then, the LRV increased with feed concentration between 10^6 and 10^8 viruses.L^{-1}. For concentrations higher than 10^8 viruses.L^{-1}, MS2 phages removal showed similar results than the CV-B5 experiments at high concentrations with a stable 3.0 log removal. Differences between the removal efficiencies of the three viruses need to be considered. For ADV 41 and CV-B5 retention, the increasing of LRV, when feed concentration of viruses increases, followed the same tendency (Fig. 1 a-b). Between 10^3 and 10^8 viruses.L^{-1}, the MS2 phages retention was lower than the two other viruses.

Table 4
Viral feed concentrations (in viruses.L^{-1}) corresponding to different concentration levels designated in membrane ageing filtration experiments.

Concentration level	ADV 41	CV-B5	MS2 phage
High	> 10^8	> 10^8	> 10^8
Intermediate	–	10^4	–
Low	10^2–10^3	10^2–10^3	10^3–10^4

According to size exclusion mechanism, retention of ADV 41 should be higher than the two others, while MS2 and CV-B5 retention should be similar (about 100 nm vs 30 nm). These results confirmed that size exclusion regarding the size of individual viruses was not a predominant mechanism for retention of such viruses with this membrane at low concentrations. This was in agreement with other studies which concluded on a small contribution of size exclusion for experiments presenting similar size between pores and viruses [43]. Then, size exclusion as the main factor leading to retention is only consistent for membranes with lower pore size than retained components [6,9,17]. With a low size exclusion contribution, viruses removal by UF are then complex and depend on many mechanisms as hydrophobic and electrostatic interactions [12,20]. In this study, both membrane [50] and viruses present negative net charge at neutral pH [27,51,52], leading to electrostatic interactions. Hydrophobicity of viruses compared to the hydrophilic membrane surface [50] prevent hydrophilic adsorption [11]. Thus, basal retention of 1 log at the lowest concentrations could be mainly govern by electrostatic and/or hydrophobic interactions. Moreover, no membrane fouling by virus adsorption or pore blockage was observed. Indeed, filtrations conducted at constant flux reported no TMP increase during filtration (data not shown). Despite evidence of hydrophobic and electrostatic interactions leading to retention of the three studied viruses, differences of removal for the three viruses were still complex to explained. Indeed, charge and hydrophobicity of viruses are dependent of numerous characteristics as isoelectric point of the component (pI) and pH of the solution [27,51], but also on the genome size [17] or the nature and concentrations of ions in solution [53–55].

The mechanisms leading to the relation between feed concentration and UF performance were also complex to understand. Few studies observed increase of retention with feed concentrations, but this phenomenon was associated with filtration flux decrease because of pore blocking [56–58], which was not consistent with the present study. Liu et al. have shown a recovery enhancement of MS2 and ϕX174 phages with increasing feed concentrations [10]. Phages recoveries at higher feed concentrations higher than 100% were then attributed to disaggregation of viruses during filtrations, introducing the hypothesis of

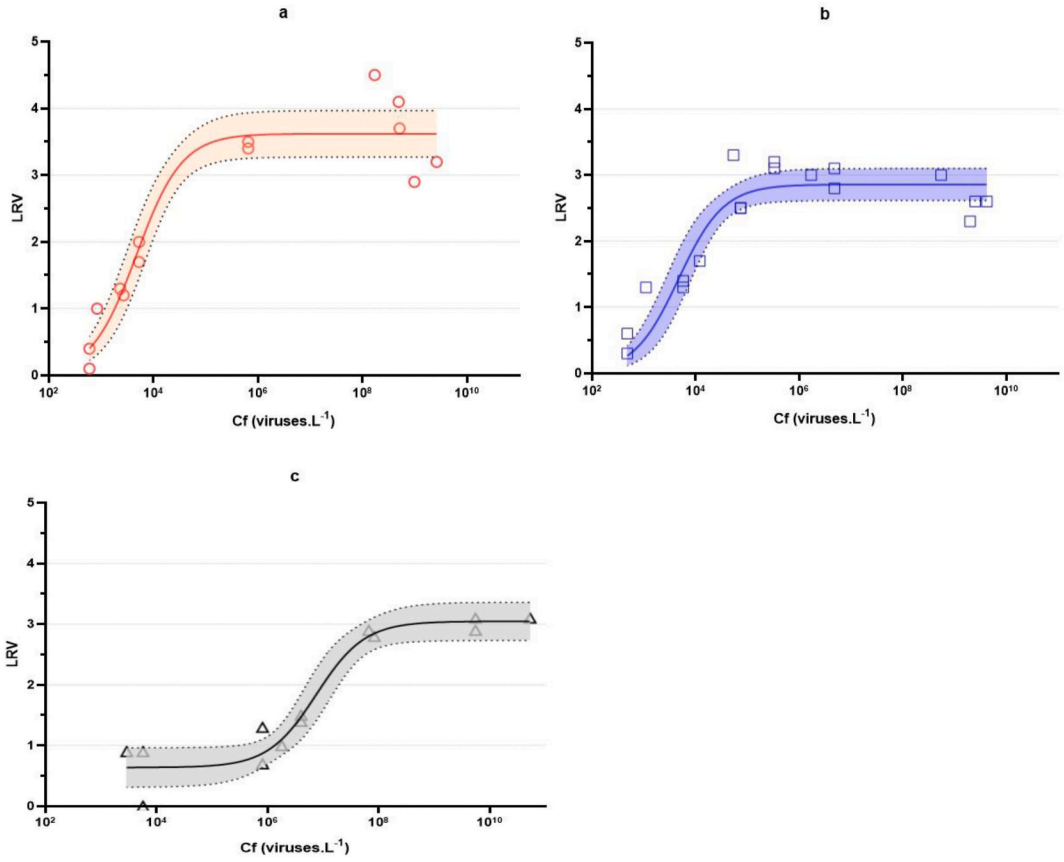

Fig. 1. Variation of LRV as the function of the virus concentration for (a) ADV 41 (b) CV-B5 (c) MS2 phage. [constant flux 100 L h^{-1} m^{-2}; VCF = 450]. Shadow areas represent a confidence level of 95%. Each point represents a single experiment.

viral aggregation in the feed solution. The aggregates in the feed lead to wrong calculation of feed concentrations with the plaque assay [17] and explained the recoveries higher than 100%. The RT-qPCR method used in this study allowed analysis of genomic copies instead of infectious units and avoid confusion between aggregates and isolated viruses' enumeration. The analyses differences between the pfu method and the RT-qPCR could be explained by the aggregates causing wrong enumeration with the plaque assay, or the detection of genomic copies of non-infective viruses with the RT-qPCR [28]. In the present study, the use of integrity assay (i.e. EMA treatment) allowed to take account on the disruption of virus capsid during the filtration process, leading to inactivation of viral particles but that could remain detectable in permeate by RT-qPCR. The hypothesis of influence of viral concentration on the stability of the virus suspension and the formation of viral aggregates could then explain the LRV increase. Viral aggregation is complex and depends on various factors as pH [59], composition of the solution [26,54,60], virus characteristics. The two major parameters reported in studies influencing viral aggregation are the pH and the ionic strength. In the conditions of this study, experiments were done with close to neutral pH at low ionic strength (10 mM), which are reported to prevent aggregation [17]. However, other parameters could impact this phenomenon. As an example, conditions of viral stock culture and dilution of viruses, can also lead to viral aggregation [61]. The impact of concentration on aggregation present difficulties of study because of the limit of detection of analysis methods. Moreover, efforts to characterize viral aggregation are done in bulk solution, but the behavior of the viruses suspension in the area of the membrane surface during filtration process could also be different [55]. Indeed, in bulk solution, electrostatic repulsions between viruses occur and inhibit the aggregation formation. Close to the membrane surface, interactions between viruses and membranes are added and the balance between virus-virus repulsions and virus-membrane interactions are difficult to predict and study. Studies attributed the different removals of different viruses on the virus characteristics (hydrophobicity, genome size etc.) influencing the virus-membrane interactions [17,28,55]. The hypothesis of viral aggregation depending on concentration and nature of the virus could explain the filtration mechanisms differently: the characteristics of the different virus as hydrophobicity and charge would influence the virus-virus interactions and their capacity to aggregates. The formation of aggregates would then explain in the meantime (i) the influence of concentrations on retention and (ii) differences between retentions of various viruses. The hypothesis of increase of aggregation with viral concentration in feed is consistent with the presented results. At high concentration, aggregates were formed and lead to a better size exclusion with aggregates much larger than pore sizes. Aggregates formation could also modify the virus-membrane hydrophobic and electrostatic interactions if charge and hydrophobicity of aggregates were different of isolated viruses [55,62]. Despite the lack of evidence of viral aggregation with increasing concentration, this assumption is consistent to explain the increase of LRV with the feed concentration. Further investigations could be done to evaluate this aggregation both in bulk solution and near the membrane surface with varying virus concentrations. However, actual analytical methods usually used (flow cytometry, size distribution, electronic microscopy) usually require high concentrations or are not applicable to the small size viruses.

These results were in agreement with the study lead by Jacangelo et al. who also analyzed the effect of feed concentration with UF retention with MS2 phage and observed the stability of UF removal efficiency for high concentrations but only for 2.0 log removal of MS2 phages [9]. These lower removals can be explained by the larger MWCO (500 kDa), compared to our results using a membrane having a 200 kDa MWCO. The impact of membrane characteristics as hydrophobicity,

charge and particularly MWCO on retention has already been proven in the same study and explain the differences of MS2 phages retention in these two studies [9]. Many other conditions can also influence UF removal efficiency, such as filtration transmembrane pressure [18] or water quality. Similarly, the retention of MS2 phages in the experimental conditions (frontal filtration, constant flux 100 $L.h^{-1}.m^{-2}$) of our experimental set up showed a maximum of 3.0 log removal, which is 1 log lower than the 4.0 log removal provided by membrane producers (tangential filtration, flux unknown). The differences between tangential and frontal filtrations [63] and TMP/flux of filtration are two main factors influencing the retention and attention must be kept on experimental conditions for comparison between retention efficiencies.

Concerning the use of MS2 phages as enteric viruses surrogates, retention of MS2 phages in this study was lower than CV-B5 and ADV 41 retention for concentrations below 10^8 viruses.L^{-1} and similar to CV-B5 (i.e. lower than ADV 41 retention) above this limit. MS2 phages could therefore be considered as suitable surrogates representing the "worst case" retention efficiency for enteric viruses. According to removal mechanism described here, MS2 phages would then be considered as surrogates only for viruses presenting similar surface characteristics as pI [27] and hydrophobicity. Globally, impact of viral feed concentration on LRV showed differences between UF performance according to the virus concentrations in raw water. Better retentions were observed for high concentration experiments. These results showed that laboratory experiments, often assessed with artificially high concentrations of viruses present a risk of overestimation of UF performances for removing virus in real industrial conditions in DWTP [55], and could induce a wrong feeling of safety when using membrane for disinfection purpose.

3.2. *Effect of water*

Filtrations experiments results obtained with raw groundwater and Evian water (laboratory-used water) were compared considering the virus feed concentration. ADV 41 experiments showed similar retention with both waters with 0.5–1.0 log removal at the feed concentration used (Fig. 2a). Experiments with CV-B5 and MS2 phages showed either similar or higher retention with groundwater compared to Evian water (Fig. 2b and c). It should be noted CV-B5 LRV represented with a vertical arrow for groundwater experiment (Fig. 2b) stand for minimal LRV calculated based on analysis detection limits for permeates with undetected viruses. Only a small effect of the water matrix is observed here, with a small tendency of better retention with the groundwater. Studies have shown water characteristics can influence the viral retention either by membrane fouling or modification of electrostatic and/or hydrophobic interactions between viruses and membrane surface. First, "low quality" waters typically with high turbidity and organic matter can cause membrane fouling, with pore clogging, pore size diminution and formation of a layer cake, which can enhance the viral retention [64]. Then, electrostatic and hydrophobic interactions can be influenced by various water characteristics as pH, ionic strength, type of ions in solution or turbidity [8,25,65,66]. Dishari et al. observed optimal conditions for viral removal with low pH and low ionic strength [65]. When the pH is lower, close to the isoelectric point of viruses, the negative surface charge is minimized, leading to a higher probability of viral adsorption on membrane. Natural Organic Matter (NOM) in solution can also influence the virus-membrane interactions. Jacangelo et al. studied the MS2 phages retention with three different water qualities and observed better removal efficiencies with increasing turbidity and TOC [9]. Madaeni et al. also concluded on the retention increase with biomass content and water turbidity [67]. Waldman observed interactions of viruses with NOM and hypothesized the formation of NOM-virus aggregates [68]. These interactions could also modify the retention mechanisms. In the present study, water characteristics of the two studied waters were very similar (Table 3) with close to neutral pH, low turbidity and low ionic strength. The groundwater presented a slightly higher TOC (0.64 mg.L^{-1} compared to $<$ 0.20 mg.L^{-1} for Evian water) and the two waters have different compositions of ions types and concentrations despite a similar global conductivity and ionic strength.

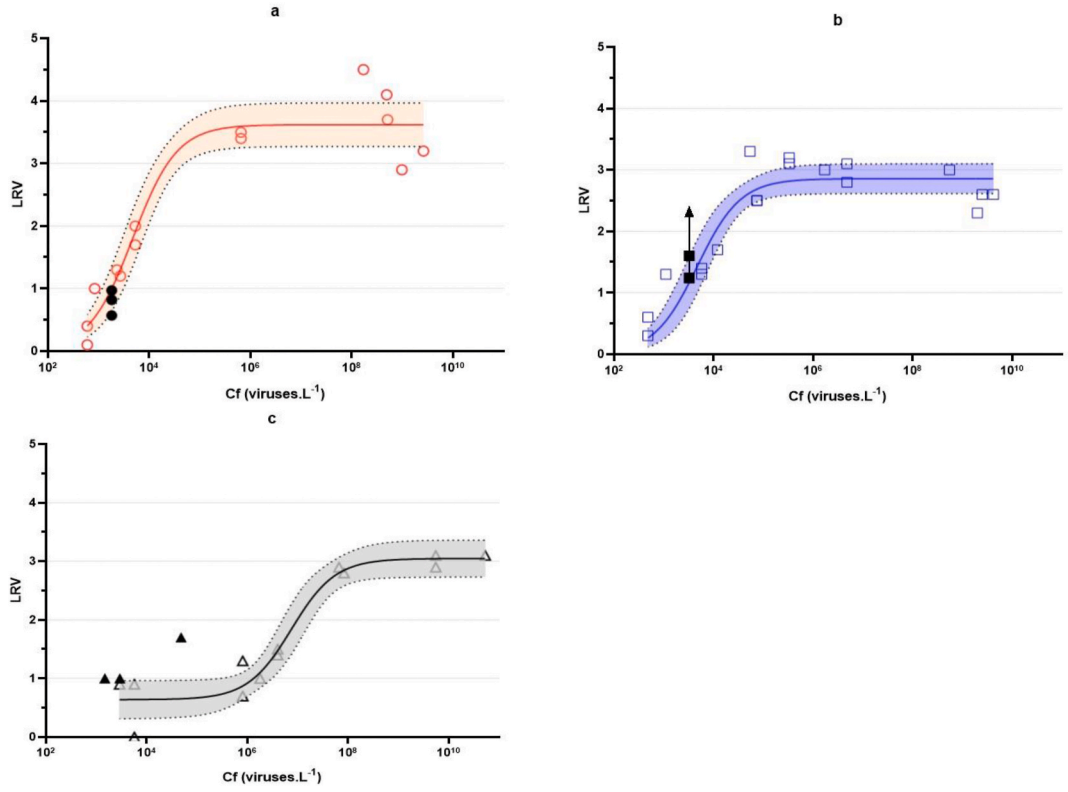

Fig. 2. Variation of LRV as the function of the virus concentration for (a) ADV 41 (b) CV-B5 (c) MS2 phage. [constant flux 100 L h^{-1} m^{-2}; VCF = 450] with different matrices ((○, □, Δ) for Evian water, (●, ■, ▲) for groundwater)). Shadow areas represent a confidence level of 95%. Each point represents a single experiment.

The differences in ion type and TOC values could possibly explain the slightly higher removal for CV-B5 and MS2 phages experiments with groundwater. Globally, experiments with Evian water presented similar or lightly smaller viral retention efficiencies, making results in laboratory conditions consistent with industrial conditions. The laboratory results with Evian water presented no health sanitary risk because virus removal efficiency could be underestimated but overestimation is avoided.

3.3. Membrane ageing

Evolution of membrane ultrapure water permeability against chlorine exposure dose (Fig. 3) showed an increase in membrane permeability after immersion in chlorine. A fast increase for the first exposure doses was observed, with a factor 2 between the membrane exposed to 6 000 $mgCl_2.h.L^{-1}$ (2 000 $L.h^{-1}.m^{-2}.bar^{-1}$) compared to the native membrane. The rise then decelerated up to a water permeability of 2 550 $L.h^{-1}.m^{-2}.bar^{-1}$ after 45 000 $mgCl_2.h.L^{-1}$ exposure dose. This increase of permeability after static immersion in chlorine was consistent with other membrane ageing studies [29,34,35,46,69–74]. It is interesting to note that membrane ageing studies with fouling/cleaning cycles lead to a decrease in permeability because of an increase in irreversible fouling with cycles [30,37,75,76]. In both cases, evolution of permeability after membrane exposition to chlorine was a sign of modifications of membrane material. The main modification reported was the degradation of PVP additives in membranes [69]. These alterations of membranes could lead to various structural changes as pore size enlargement/reduction and modification of surface rugosity [31,35,39,46] or hydrophobicity [31]. These changes in membrane properties could lead to changes in retention performances. Studies already observed different results depending on membrane modifications and retention compounds. Ravereau et al. showed an increase in MS2 phages retention after membrane exposition to chlorine because of the increase in hydrophobicity of membrane surface [40]. Studies about retentions of bovine serum albumin (BSA) concluded on the enhancement of BSA retention with increase of membrane hydrophilicity, or BSA retention reduction with increase in membrane hydrophobicity [30,34,35]. These results showed that depending on the membrane material, ageing with chlorine exposure can lead to opposite retention modifications. Permeability was reported as a good indicator of membrane structural changes with ageing, and particularly easy to follow in industrial conditions. Retention performances against viruses are still difficult to predict and attention must be paid on their evolution with time.

According to evolution of LRV with feed concentrations for native membrane, retention of viruses with membrane ageing has been assessed with different feed concentrations (Table 4). Concerning the evolution of LRV depending on the chlorine exposure dose, ADV 41 retention showed different results at high and low feed concentrations (Fig. 4a). For low feed concentrations (○), the membrane exposition to chlorine showed no significant impact on the retention efficiency ($p > 0.05$), while the retention for experiments at high concentrations (●) showed a significant decrease in retention for membranes exposed to 11 000 $mgCl_2.h.L^{-1}$ compared to native membranes ($p = 0.034$). Differences between the ADV 41 retentions at high and low feed concentrations with membrane ageing confirmed the problem of comparison of studies at different concentrations. For CV-B5 and MS2 phages experiments (Fig. 4b–c), no significant difference of retention between native and aged membranes was reported for all feed concentrations considered ($p > 0.05$). For low concentrations retentions, membrane modifications with chlorine exposure, highlighted by increasing of permeability, did not influence the retention of viruses. Other studies already related the membrane ageing to modification of pore size distribution, rugosity, hydrophobicity, but these phenomena depend on the membrane material and the ageing conditions. Evaluation of the membranes changes with membrane ageing in the conditions of these studies, in parallel with virus retention evolution could allow a better understanding of retention mechanisms. Moreover, other authors have noticed different removal of MS2 phages at full scale between native and aged membranes [25]. Despite the evidence of membrane modifications with chlorine ageing, it is not responsible of these removal efficiency changes. Then, effect of other parameters (i.e. fouling, backwash, other cleaning solution) should be investigated.

The use of MS2 phages as virus surrogate has been discussed for native membranes, and results have shown that MS2 phages could be a suitable surrogate as “worst case” for CV-B5 and ADV 41. Concerning the evolution of retention for membrane ageing, for low feed concentrations, the three viruses didn’t show any evolution. MS2 phage could therefore be considered as a suitable surrogate for retention evolution follow with membrane ageing with low feed concentrations. However, for high feed concentrations, ADV 41 retention decreased with membrane exposition to chlorine, while MS2 phages and CV-B5 retentions were stable. In these conditions, MS2 phages were not a suitable surrogate for ADV 41 retention.

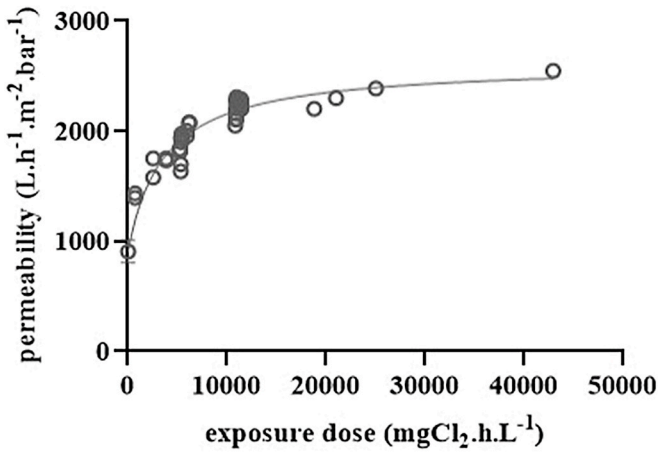

Fig. 3. Variation of permeability as the function of the chlorine exposure dose ($mgCl_2.h.L^{-1}$) after static immersion [30 $mgCl_2.L^{-1}$; pH 7.5].

4. Conclusion

UF for virus removal has been assessed regarding various experimental conditions and comparison between laboratories studies and DWTP conditions has been studied. Laboratory studies mostly use high virus feed concentrations, while resources in DW production are largely less concentrated. Study about the effect of feed concentration on native membranes showed a better removal efficiency for highest feed concentrations. These results highlighted that high feed concentrations studies present a risk of overestimation of the UF performances with retentions around 3.0 log. In low feed concentrations ($< 10^3$ viruses.L^{-1}) UF achieved retentions lower than 1.0 log removal for all viruses studied (ADV41, CV-B5 and MS2 phages). These higher retentions at high concentrations could be explained by formation of viral aggregates, which could facilitate the steric exclusion but also modify the electrostatic and hydrophobic interactions between isolated viruses/aggregates and membrane. The impact of water on native membranes has been evaluated, resulting in a similar or better removal efficiency with groundwater from DWTP (Eau de Paris, Saint-Cloud DWTP, France) compared to Evian water, used for laboratory experiments. Finally, membrane ageing caused by chlorine has been assessed regarding membrane water permeability and virus removal. An increase of membrane water permeability from 1 000 $L.h^{-1}.m^{-2}.bar^{-1}$ (native membranes) up to 2 500 $L\ h^{-1}\ m^{-2}.bar^{-1}$ (45 000 $mgCl_2.h.L^{-1}$ chlorine exposure) revealed membrane degradations. A fast increase up to 2 000 $L.h^{-1}.m^{-2}.bar^{-1}$ for 10 000 ppm.h was observed, and then a slower increase. This ageing of membrane material was therefore not influencing the viral removal for low feed concentrations. The permeability follow for membrane ageing

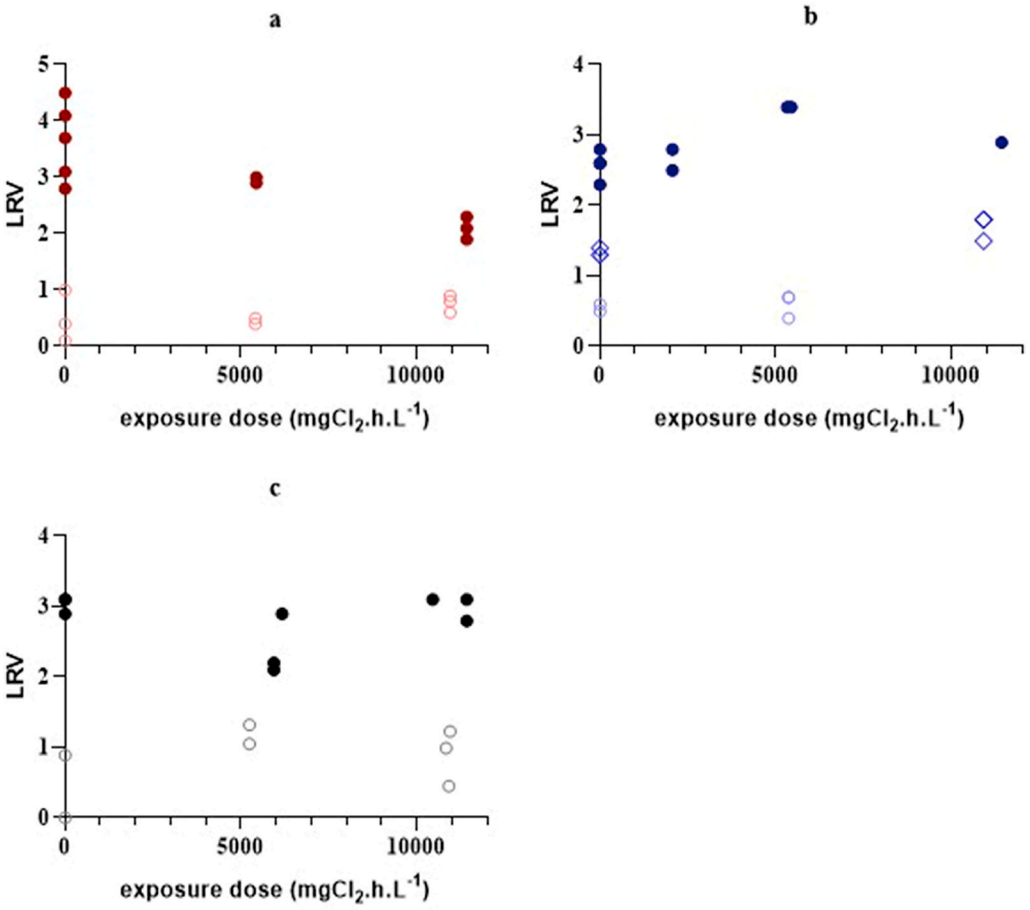

Fig. 4. Variation of LRV as the function of the chlorine exposure dose ($mgCl_2.h.L^{-1}$) after static immersion [30 $mgCl_2.L^{-1}$; pH 7.5] regarding retention of a) ADV 41 b) CV-B5 c) MS2 phages, at various feed concentrations ((●) for high feed concentrations, (◇) for intermediate feed concentrations, (○) for low feed concentrations). Each point represents a single experiment.

evaluation was therefore not representative of viral removal performances. Thus, differences between laboratories and industrial conditions still exist, and further investigations on repeated filtration/cleaning experiments could evaluate the evolution of virus removal with cycles. The impact of backwash, other cleaning agents and various industrial conditions should be assessed by challenging real end-of-use membranes from DWTP after several years of use. Furthermore, removal comparison of two enteric viruses (ADV 41 and CV-B5) with the MS2 phage surrogate showed that these surrogate fates were similar or lower than the two studied enteric viruses, making the MS2 phage suitable as a "worst case" virus surrogate.

Author statement

N. Jacquet Investigation and writing.
Y. Wyart Co supervision.
S. Wurtzer Investigation and analyses.
L. Moulin Resources, industrial partenair
G. Darracq Investigation and writing.
P. Moulin Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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