Food And Environmental Virology
March 2024, Volume 16 Issue 1 Pages 14-24
https://doi.org/10.1007/s12560-023-09574-z
https://archimer.ifremer.fr/doc/00869/98136/

Retention of Virus Versus Surrogate, by Ultrafiltration in Seawater: Case Study of Norovirus Versus Tulane

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Abstract :

In the field of chemical engineering and water treatment, the study of viruses, included surrogates, is well documented. Often, surrogates are used to study viruses and their behavior because they can be produced in larger quantities in safer conditions and are easier to handle. In fact, surrogates allow studying microorganisms which are non-infectious to humans but share some properties similar to pathogenic viruses: structure, composition, morphology, and size. Human noroviruses, recognized as the leading cause of epidemics and sporadic cases of gastroenteritis across all age groups, may be mimicked by the Tulane virus. The objectives of this work were to study (i) the ultrafiltration of Tulane virus and norovirus to validate that Tulane virus can be used as a surrogate for norovirus in water treatment process and (ii) the retention of norovirus and the surrogate as a function of water quality to better understand the use of the latter pathogenic viruses. Ultrafiltration tests showed significant logarithmic reduction values (LRV) in viral RNA: around 2.5 for global LRV (i.e., based on the initial and permeate average concentrations) and between 2 and 6 for average LRV (i.e., retention rate considering the increase of viral concentration in the retentate), both for norovirus and the surrogate Tulane virus. Higher reduction rates (from 2 to 6 log genome copies) are obtained for higher initial concentrations (from 101 to 107 genome copies per mL) due to virus aggregation in membrane lumen. Tulane virus appears to be a good surrogate for norovirus retention by membrane processes.

Keywords : Norovirus, Tulane virus, Virus surrogate, Ultrafiltration, Retention rate, Seawater quality

1. Introduction

The risk of microbial contamination of water by waterborne enteric viruses via sewage is a major concern due to their serious adverse risks to human health as reported by World Health Organization (Hamza et al., 2009), such as acute viral gastroenteritis (Atmar et al., 2018; Woodall, 2009), acute hepatitis (Altintas et al., 2015), pneumonia and respiratory infections (Sinclair et al., 2009). Therefore, to minimise the risks associated with the extent of viral diseases, biological or physico-chemical processes such as ultraviolet (UV) irradiation and ozonation have been developed in the last decade (Lanrewaju et al., 2022). Among all the available technologies, membrane-based treatment methods have shown great advantage in removing viruses from contaminated water (Antony et al., 2012). Ultrafiltration, with a Molecular Weight Cut Off (MWCO) around 100 kDa, in particular is reported as an efficient removal treatment for many viruses and is increasingly used for drinking water production (Ferrer et al., 2015; Gentile et al., 2018). Indeed, the efficiency of ultrafiltration for virus retention has already been proven by high logarithmic reduction values (LRV): 6 log for the MSE2 bacteriophage (Jacangelo et al., 1995), > 4 log for T4 bacteriophage (Urase et al., 1996), > 6 log for poliovirus (Otaki et al., 1998), > 5.7 log for human immunodeficiency virus and pseudorabies virus (Wang, 2001), 4 log for avian influenza A (H5N1) virus (Lénès et al., 2010), 4 log for norovirus (Matsushita et al., 2013), 3.1 log for pepper mild mottle virus (Lee et al., 2017), and 3-3.5 log for adenovirus (AdV) 41 and coxsackievirus (CV) B5 (Jacquet et al., 2021). It is important to note that these retentions are obtained for high virus concentrations compared with those found in natural waters. A disparity in retention is observed but can be explained by differences in membrane cut-offs, virus concentrations and virus sizes. Although ultrafiltration is mainly based on steric exclusion phenomena, many interactions and transport mechanisms coexist and contribute to the overall retention of virus.

Regarding aquaculture, the costal water quality is essential, especially when shellfish are consumed raw or slightly cooked. Indeed, shellfish (mainly oysters) is the food more frequently implicated in foodborne outbreaks despite the set-up of regulation based on a fecal indicator (*Escherichia coli*) either in water or in shellfish flesh and fluid (Rowan, 2023). Most of these outbreaks are due to oyster (consumed raw, mainly during the winter season) and norovirus. Noroviruses are recognized as the leading cause of epidemics and sporadic cases of gastroenteritis across all age groups. They are excreted in large quantities by ill people, but may

also be present in asymptomatic, healthy individuals (Atmar et al. 2018). As a consequence, they are discharged in high concentrations in sewage and due to their resistance to inactivation, they are frequently detected in wastewater treatment plant effluents and in surface waters (Sano et al., 2016). Un-correctly treated sewage or accidental input of raw sewage following heavy rain-fall or broken sewage pipes contributes to coastal area contamination (Maalouf et al. 2010). Shellfish, especially oysters, filtering large volumes of seawater for their physiological activities may then be contaminated by these viruses that are very stable in seawater (Desdouits et al., 2022). When oysters are contaminated, depuration (immersion of oysters in clean seawater), which is efficient to eliminate bacteria, failed to eliminate norovirus (Mc Leods et al. 2017, Younger et al. 2020). As a consequence, the only risk management option to prevent consumer infections is the closure of production areas, with viral testing of shellfish to evaluate the decrease of viral contamination.

A good surrogate must present the closest morphological and biological properties (e.g. morphology, composition, resistance to different treatments) to the target virus (Cromeans et al. 2014). Bacteriophages, easy to handle and non-infectious to humans, are often considered as a surrogate to enteric viruses due to their structure, composition, morphology, and size (Grabow, 2004). Challenge testing with bacteriophage MS2 is currently the most frequently used indicator of membrane processes efficiency in terms of virus removal (Antony et al., 2012). However, some differences limit their universal use such as the electrostatic charge which affects the adsorption phenomenon on membranes especially on charged membranes.

Human norovirus cannot be multiplied in large number in cell culture (Ettayebi et al, 2016). To study their behavior, surrogates have been proposed such as phages or viruses from the *Caliciviridae* family closely related to human noroviruses, such as murine norovirus (MNV), as it can best mimic their survival and inactivation (Frohnert et al., 2015; Hirneisen and Kniel, 2013; Wang et al., 2022). In recent years, Tulane virus has also emerged as a potential substitute for human norovirus. The Tulane virus, belonging to the Recovirus genus from the *Caliciviridae* family present several advantages for being a surrogate for norovirus (Farkas, 2015). Both viruses have a size of ~40 nm in diameter and mature, infectious virions, a short genome of about 6,714 to 7,700 nucleotides, organized in three open reading frame (ORF) (Kniel, 2014). The major structural protein (VP1) organized in 90 dimers forms the capsid, and is constituted of two distinct domains, where the shell (S) domain makes up the inner part and the protrusion (P) domain makes up the outer part of virions (Farkas, 2015). Based on the known structures, the overall fold for the S and P domains are nearly identical across all caliciviruses, where the S domain is the most conserved (Desselberger, 2019). The greatest diversity is found

at the distal end of the P domain and protein residue composition in this region is used to distinguish the different strains. To address their electronic charge, the isoelectric point (pI) of VP1 are compared across different caliciviruses. From the comparison of Tulane virus and some norovirus strains, the pI of VP1 was relatively similar with a value of 5.3. In addition, electrostatic plots of some of the known structures show that the charge on the outermost surface of noroviruses is generally neutral to slightly negative (Choi *et al.,* 2011; Farkas *et al.,* 2008; Samandoulgou *et al.* 2021). The Tulane virus was used as a surrogate to study the norovirus in oysters (Drouaz et al., 2015; Polo et al., 2018) and in comparative studies on inactivation and stability (Barnes et al., 2021; Recker and Li, 2020; Bartsch et al., 2019; DiCaprio et al., 2016, 2019).

The objective of this study was to better understand the filtration behavior and retention efficiencies of the Tulane virus and norovirus by ultrafiltration (UF) of seawater, considering the influence of the concentration on the retention.

2. Material and methods

2.1. Virus preparations

Tulane virus (TV) strain M033 (provided by T. Farkas, Cincinnati children's hospital, Cincinnati, USA) was propagated in confluent monolayers of LLC-MK2 cells (ATCC® CCL-7™, Manassas, VA) as previously described (Farkas et al., 2008). Mengovirus (MgV) strain pMC0 (provided by A. Bosch, University of Barcelona) was propagated in HeLa cells according to protocol previously described (Martin et al., 1996). When cytopathic effects (CPE) were complete, the dishes with cultures were submitted to three cycles of freezing/thawing at − 20°C. Than the cell debris were removed by centrifugation at 1,000 × g for 30 min. The supernatant, containing viral particles, was then purified and concentrated using 0.2 µm filter and Amicon ultra-15 centrifugal filters (Millipore, France).

For norovirus, a raw sewage sample with a known concentration was used. All viral inoculum (Tulane virus or raw sewage) were homogenised by vortexing, aliquoted and stored at -70°C until use (Taligrot et al., 2022). A natural seawater taken at Bourgneuf Bay (Vendée, France) was filtered (10 µm and 1 µm) and UV treated (Bio UV PE 2160 HO, equipped with 2 lamps of 87W). The seawater samples were then spiked with the prepared inoculum to obtain 480 mL of the feed solution with a final Tulane virus or norovirus concentration around 10^6, 10^4 and 10^2 genome copies per mL. The viral suspension did not exceed 10 % of total sample volume to avoid the modification of physical parameters of the seawater.

2.2.Membrane and water characteristics

Membrane modules were made of one hollow fiber placed into a PVC external shell with an epoxy plug on each side of the module. Membranes used were polymeric multichannel hollow fibers (ALTEON^{TM} I, SUEZ Aquasource®, France), made with hydrophilic polyether sulfone (PES) and a porogenic hydrophilic polymer (polyvinylpyrrolidone, PVP). Their external diameter was 4 mm and they were composed of 7 channels with an inner diameter of 0.9 mm (Lehir et al., 2018). The active length of fiber was 25.5 ± 0.4 cm which provided a specific surface of (5.04 ± 0.07) × 10^{-3} m² and an internal volume of 1.14 ± 0.02 mL. The MWCO given by the manufacturer is 200 kDa with a membrane pore size distribution centered around 20 nm. This membrane is used to produce drinking water for instance in France (Nancy, Orléans, l'Haÿ-les-Roses), Croatia (Dubrovnik), Switzerland (Lutry) or Italy (Castiglione de Fiorantino), and recently for different aquaculture applications (Cordier et al., 2018, 2019ab, 2020abcd, 2021).

After the module potting, membranes were rinsed with ultrapure water under different transmembrane pressures (TMP) with a maximum at 1.0 ± 0.1 bar to remove the preservative agent (glycerin) (Arenillas et al., 2007). Initial water permeability (Lp_0) was then measured with ultrapure water before each filtration experiment. An average ultrapure water permeability at 20°C of 714 ± 40 L h^{-1} m^{-2} bar^{-1} was obtained (i.e. membrane resistance = 5.04 x 10^{11} m^{-1}), a value in agreement with the supplier's data.

2.3. Filtration procedure

Experiments were performed in dead-end filtration mode with constant TMP: approximately 0.3-0.4 bar. To avoid any contamination between experiments, a new membrane module was used for each essay. The volume of the feed virus suspension was 480 mL. 80 mL were sampled from the initial virus suspensions to measure the feed concentration and resting 400 mL were supplied from the feed tank pressurized with air to the connected membrane module. During the filtration process, permeate was collected over time. Almost every 5 to 10 s, the weight was recorded by an electronic balance (Δm = ± 0.01 g; Mark Bell, Berlin, Germany) to calculate the permeate flux (J in $L.h^{-1}.m^{-2}$) from the permeate flowrate (Q in $L.h^{-1}$) and the membrane surface (S in m^2). The temperature was used to correct the measured flux at 20 °C in agreement with the variation of water viscosity. The permeate flux values along time allowed to visualize the potential fouling formation during filtration. The permeate samples of 80 mL were taken for analysis at the beginning (P1), in the middle (P2-P3) and at the end of filtration (P4). Thus, five

samples were taken during each filtration (feed suspension, P1, P2, P3, and P4), frozen at -20°C, and further analyzed in triplicate for virus detection.

2.4. Sample analysis

For nucleic acid extractions (NA), the process control virus, MgV was added to 60 mL of sample prior to extraction to control for virus recovery. Then the samples were centrifuged at 204,000 × *g* for 1h at 4°C (Ti45 rotor and Co-LE80K centrifuge, Beckman Coulter Life Sciences, France). The obtained pellet was resuspended in 500 µL of PBS and incubated for 5 min at ambient temperature. NA were then extracted and purified using NucliSens system (NucliSens Lysis buffer and NucliSens Extraction Reagents, bioMérieux, Lyon, France) on a semi-automatic MiniMag ramp (bioMérieux, Lyon, France) according to manufacturer's protocol. The NAs were eluted in 100 µL of elution buffer using a Thermomixer (Eppendorf) at 60°C with 1400 oscillations min^{-1} during 5 min. The RNA of MgV process control virus was extracted in parallel. Extracted nucleic acids were kept at 5°C for 48 h maximum to perform all the amplifications and then at -20°C for longer storage. For amplification, one-step real time RT-PCR (RT-qPCR) approach was applied. First, RT-qPCR MgV was run to evaluate the extraction efficiency and validate the extraction. After validation, all extracted NA were analysed in triplicates for each target viruses (Tulane virus and norovirus GI and GII performed in separate runs). The reaction mixes prepared with RNA UltraSense One-Step Quantitative RT-PCR System, (Invitrogen) contained a final concentration of 500 nM of forward primer, 900 nM of reverse primer and 250 nM of probe. The primers and probes used in the study were as described previously for the Tulane virus (Drouaz et al, 2015) and for norovirus and MgV (ISO 15216-1:2017). The absence of inhibitor was verified by adding synthetic RNA of external control (EC) carrying the target sequence as described in ISO 15216-1:2017 (for Tulane virus the EC used for norovirus GI was added to the extract).

These quality controls were used to ensure the quality of the assays:

(I) *Extraction efficiency*. Virus recovery was controlled according to protocol described in ISO 15216-1:2017. To estimate the extraction efficiency, the MgV RT-qPCR results were used as follow: Process control virus recovery = $10(\Delta Cq\ m^{-1}) \times 100$ % (where ΔCq = Cycle of quantification (Cq) value (sample RNA) – Cq value and m = slope of the process control virus RNA standard curve).

(II) *Control of inhibitors*. Amplification efficiency was controlled according to protocol described in ISO 15216-1:2017. The result obtained for the EC served to calculate the RT-

qPCR inhibition = (1 − 10(ΔCq m^{-1})) × 100 % (where ΔCq = Cq value (sample + EC RNA) − Cq value (water + EC RNA) and m = slope of the dsDNA standard curve)

(III) *Amplification controls*. Positive amplification control (synthetic dsDNA of each target virus as described below) and negative amplification controls: negative extraction control – extraction on molecular grade water instead of the sample) and negative amplification control (molecular grade water instead of sample RNA) were included in each run to exclude potential falls positive and negative results.

After verification of these different controls, the number of norovirus or Tulane virus genome copies were calculated using standard curves included in each run. The dsDNA carrying a target sequence corresponding to nucleotides 3300 to 4299 of Tulane M33 virus (GenBank acc. N° EU391643.1), nucleotides 4484 to 5668 of Norwalk virus (GenBank accession number M87661) for norovirus GI and the sequence between nucleotides 4217 and 5355 of the Houston virus (GenBank accession number EU310927) for norovirus GII were synthetized by gBlock (Integrated DNA Technologies, France). The standard curves were elaborated using ten-fold serial dilutions including concentrations from 4.98 log of genome copies μL^{-1} to 0.98 log of genome copies μL^{-1} for Tulane virus; 4.91 log of genome copies μL^{-1} to 0.91 log of genome copies μL^{-1} for norovirus GI and concentration from 4.85 log of genome copies μL^{-1} to 0.85 log of genome copies μL^{-1} for norovirus GII, each concentration tested in duplicates. Calculation of standard curve parameters (e.g. slope, intercept, amplification efficiency) was performed according to NF U47-600-2:2015 European norm.

Calculation of biases linked to the standard curves and determination of the expanded linearity uncertainty. The expanded linearity uncertainty U_{LIN} was calculated according to NF U47-600-2 norm, using the following equation:

$$U_{LIN}\sqrt{\sum \frac{{U_{Link}}^2}{K}}$$

where U_{LINk} is a linearity uncertainty for each concentration level, calculated as follows:

$$U_{LINk} = \sqrt[2]{s^2 + (\overline{concentration\ of\ k\ level - theoretical\ concentration\ for\ k\ level})^2}$$

and s – standard deviation of biases

Reproducibility of the assays was evaluated for each initial concentration using MAD approach (median of the absolute deviation to the median) by application of Algorithm A (Huber's method) according to Annex C, clause C.3.1 in ISO 13528:2015. The results were judged satisfactory in term of reproducibility when a difference between a single result and results'

234 average (absolute value) were < 2SD; questionable when a difference was ≤ 3SD and
235 unsatisfactory when a difference between a single result and results' average was > 3SD.
236
237 **2.5. Treatment of the virus retention as a function of the virus concentration**
238 RT-qPCR analysis allowed to quantify the concentration of viruses contained in feed and
239 permeate samples and evaluate the virus retention efficiency by calculating the LRV, depending
240 on Cf and Cp (in genome copies mL^{-1}), the concentrations of viruses in feed, in the retentate
241 and permeate respectively:

242 $$Global\ LRV = log_{10}\left(\frac{C_f}{C_{gp}}\right) and\ average\ LRV = log_{10}\left(\frac{C_r}{C_p}\right)$$

243 where C_r, C_f, C_p and C_{gp} are respectively the concentration in the retentate, in the feed, in the
244 permeate, in the global permeate.
245 Data of Logarithmic Reduction Value (LRV) as a function of virus concentration in the feed
246 suspensions were statistical analyzed with the software GraphPad Prism 9.0. Best fit models
247 were determined for each set of data, considering each Y replicate as individual value.
248
249 **3. Results and discussion**
250 The influence of virus concentration was tested respectively with three different norovirus and
251 Tulane virus concentrations in the feed between 2.94 and 4.16×10^5 genome copies mL^{-1} and
252 0.36 and 1.09×10^5 genome copies mL^{-1} respectively. For each concentration three replicates
253 were filtered and quantified.
254
255 **3.1. Quality control of the assays**
256 The assays were organised in nine analytical series, containing the feed (P0) and permeate
257 samples (P1 - P4) for each initial concentration and replicate. A particular attention was paid
258 on reliability of the results and the assays were controlled and validated at every step of
259 analysis. First, the process control virus (MgV) served to evaluate the virus recovery and the
260 extraction was considered only if virus recovery was ≥ 1%. All experiments provided a
261 satisfactory recovery, with an extraction efficiency mean value of 55 % (from 30 to 100 %)
262 for Tulane virus and 71 % (from 29 to 100 %) for norovirus (data not shown). Then, the
263 absence of inhibitors was verified for all samples included in the amplification run. These
264 amplification efficiency control served as a quality assurance parameter only and was not used
265 to adjust the results. Finally, to avoid bias in quantification linked to standard curves, criteria
266 of acceptance were applied to (i) Cq values obtained for most concentrated point of standard

curves which needs to be included between ± 0.5 of expected Cq value for this concentration. (ii) The values of a slope for the Tulane virus were included between -3.41 et -3.10; norovirus GI standard curves were included between -3.37 and –3.19 and for the norovirus GII curves between -3.43 and -3.10. These values correspond to amplification efficiency from 96 % to 110 % for Tulane and norovirus GII and from 96 % to 106 % for norovirus GI, fulfilling the criteria of the norm U47-600-2:2015 which preconise the slope values from -4.115 and -2.839 which correspond to PCR efficiency from 75 % to 125 %, as well as ISO 15216-1:2015 norm which is stricter and preconise the efficiencies ranged from 90 % to 110 % for the slopes included between -3.6 and -3.1. A Pearson's correlation coefficients calculated for each single standard curve were included between 0.9979 and 0.9990 for Tulane virus, from 0.9977 to 0.9996 for norovirus GI and between 0.9888 and 0.9985 for norovirus GII, and thus conform to ISO 15261-1:2017 criteria ($0.980 < r^2 < 1.000$). For the norovirus, calculation of biases and determination of the expanded linearity uncertainty were then performed according to NF U47-600-2:2015 norm. Results obtained for both target virus are presented in a Tables 1 and 2.

Table 1. Expanded biases and uncertainties for norovirus GI by concentration level

Theoretical concentration (log genome copies μL^{-1})	4.91	3.91	2.91	1.91	0.91
Calculated concentration (log genome copies μL^{-1})	4.90	3.92	2.92	1.90	0.91
Biases mean (log genome copies μL^{-1})	-0.01	0.01	0.01	-0.01	0.00
Standard deviation of biases	0.04	0.03	0.03	0.02	0.03
U_{LIN} (log genome copies μL^{-1})	0.086	0.068	0.067	0.047	0.068

Table 2. Expanded biases and uncertainties for norovirus GII by concentration level

Theoretical concentration (log genome copies μL^{-1})	4.85	3.85	2.85	1.85	0.85
Calculated concentration (log genome copies μL^{-1})	4.84	3.84	2.89	1.84	0.84
Biases mean (log genome copies μL^{-1})	-0.01	-0.01	0.04	-0.01	-0.01

Standard deviation of biases	0.05	0.04	0.09	0.10	0.10
U_{LIN} (log genome copies μL^{-1})	0.099	0.078	0.204	0.205	0.194

The U_{LIN} expanded linearity uncertainty calculated from five concentration level standard curve was 0.07 log genome copies μL^{-1} for norovirus GI and 0.17 log genome copies μL^{-1} for norovirus GII. These values are conformed to NF U47-600-2:2015 requirement which calls for a tolerance of ≤ 0.25 log genome copies μL^{-1}. Reproducibility of the assays was determined by MAD approach for each initial norovirus (GI + GII) concentration. The MADs were corrected by a factor of 1.4826 to produce normalized MADs, equal to 1 for a reduced cantered normal distribution. All MAD shown in the graphs are standardized (Figure 1).

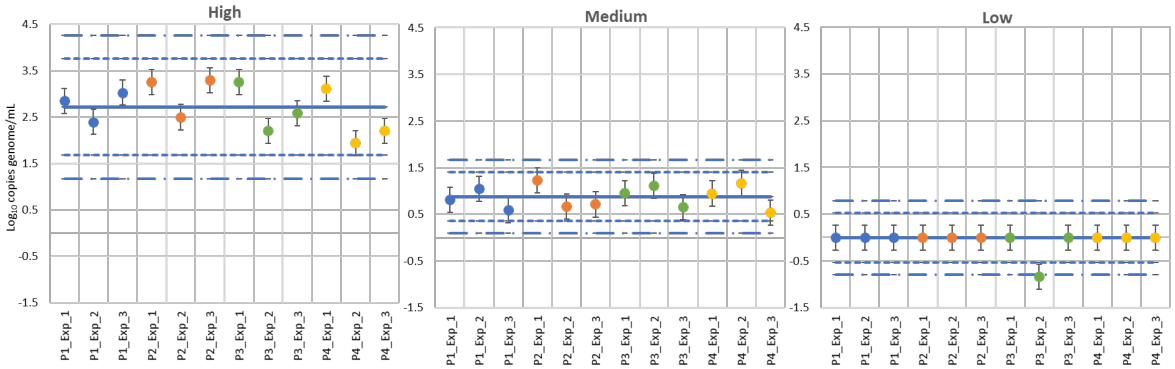


Figure 1. Reproducibility of the assays according to MAD approach for high middle and low initial norovirus concentration in the feed. Blue dots – feed samples; brown to black dots – permeate samples taken along the filtration process; ex. P1_Exp1: Permeate 1from Experiment n°1

The results show a high reproducibility. For the lowest concentrations, where the amplifications were performed twice, only one result was judged as doubtful.

3.2. Ultrafiltration of Tulane virus and norovirus: permeate flux and fouling resistance

During both filtrations of Tulane virus and norovirus, the permeability decreases as the function of the volumetric concentration factor (VCF, i.e. time) (Figure 2). The fouling differences observed between the targets can be explained by samples' preparation (see Material and methods section). Indeed, norovirus were obtained in a water contaminated using raw sewage (carrying organic matter) in contrast to Tulane virus issued from a cell culture purified supernatant. Table 3 gives the additional fouling resistance for a VCF of 300, which was the VCF reached at the end of each experiment. For Tulane virus, even for high concentrations, the

additional resistance remained low (< 1/2 of the membrane resistance). On the contrary, for norovirus, the additional resistance increased with the viral concentration and exceeded the membrane resistance when concentrations in the feed reached 10^6 viruses mL^{-1}.

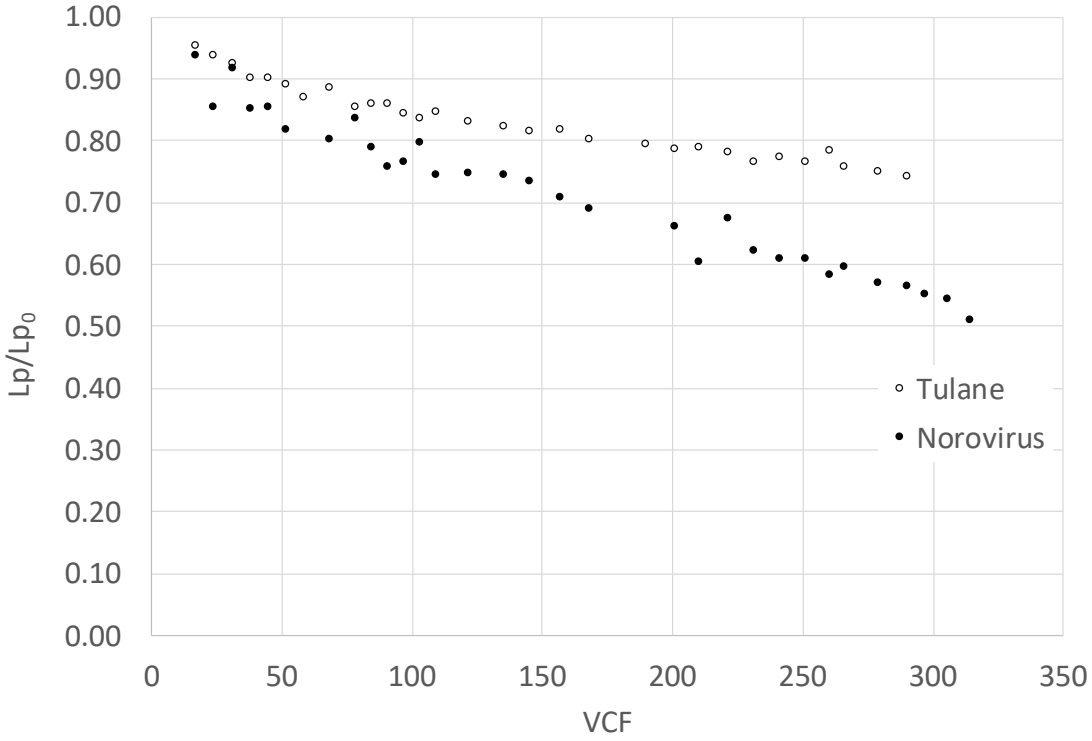


Figure 2: Variation of the dimensionless permeability as the function of Volumic Concentration Factor (VCF) for Tulane virus and norovirus; Lp – Membrane permeability; Lp_0 – Initial membrane permeability

Table 3: Additional fouling resistance (m^{-1}) as the function of the feed virus concentration for Tulane virus and norovirus (VCF = 300)

Tulane	Feed virus concentration (genome copies mL^{-1})	[42,000-109,000]	[23-525]	[0.36-1.89]
	Fouling Resistance (m^{-1})	2.3×10^{11}	2.6×10^{11}	2.2×10^{11}
Norovirus	Feed virus concentration (genome copies mL^{-1})	[201,000-294,000]	[503-2520]	[4.16-6.75]
	Fouling Resistance (m^{-1})	5.7×10^{11}	3.4×10^{11}	1.8×10^{11}

3.3. Comparison of norovirus and Tulane virus retentions by UF

The retention efficiency was determined using two retention rate calculations because only average permeates (3 in number: initial, medium and final permeates), although representative

of all permeates, were obtained for each experiment. First, the global retention was calculated based on the initial and permeate average concentrations, which did not consider the increase of virus concentration upstream of the membrane over time. This retention rate was calculated by drinking water producers for the same reasons of non-accessibility of the real concentration in the lumen (concentrate side) or the variation of permeate concentration with time. Secondly, average retention rate (i.e. average LRV) was calculated considering the three permeate concentrations obtained and the calculated median concentrations of retentate corresponding to collection times (i.e. for these three permeates). This retention rate is closer to reality as it consider the increase of viral concentration in the retentate. However, it assumes that the permeate concentration remains constant for the duration of each permeate sample. The concentration and the global LRV for Tulane virus and norovirus calculated for each filtration are compared in Figures 3 and 4. Regarding the treated water, Tulane virus and norovirus were detected in permeates thus UF does not lead to a total retention of these microorganisms except for the smaller virus concentrations in the feed (Figure 3). In this case, if the quantification in the permeate is lower than the detection limit, the virus concentration was substituted to the lowest value that can be determined i.e. 0.114 and 0.286 genome copies mL^{-1} for lowest ranges of feed concentration of norovirus and Tulane respectively.

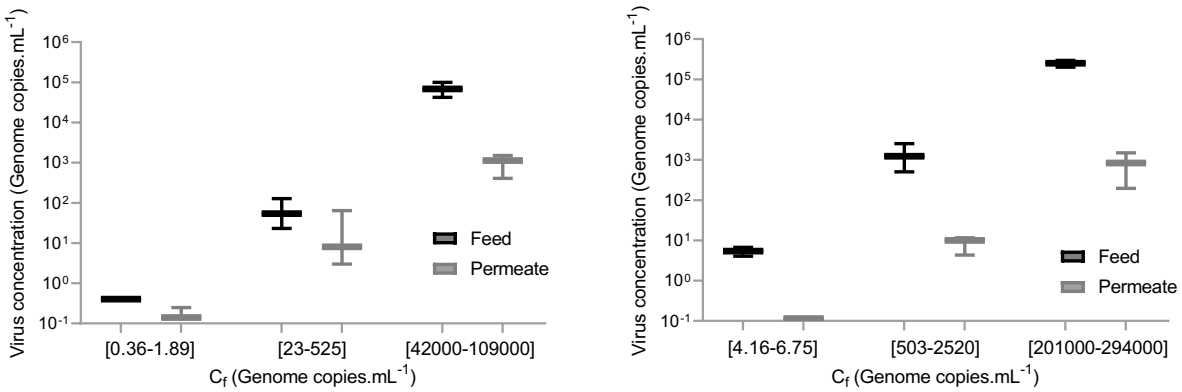
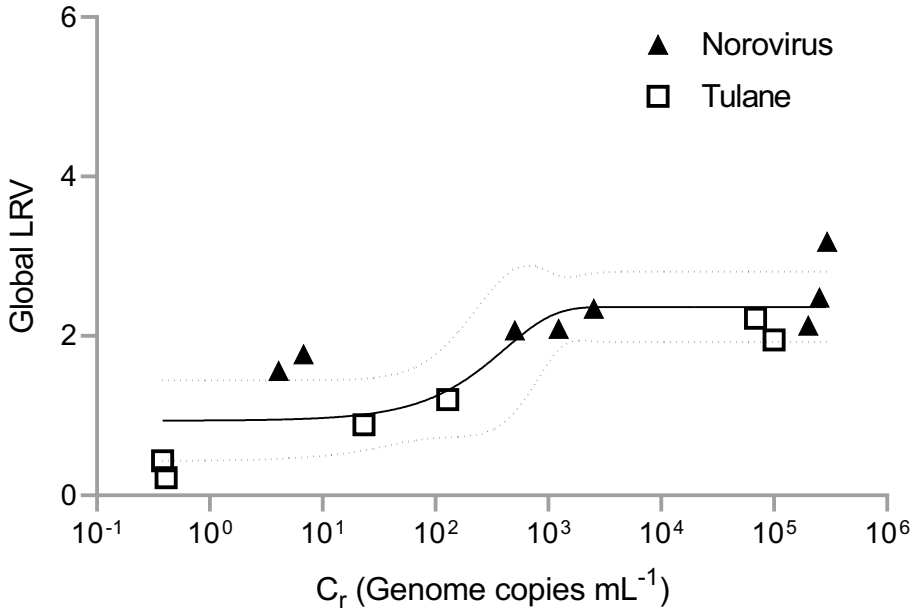


Figure 3: Variation of the feed and permeate concentrations as the function of the feed concentration for Tulane virus (left) and norovirus (right). C_r -Retentate concentration

For global LRV (Figure 4) and for the lowest feed of norovirus concentrations tested, LRV of less than 1 was obtained. With the feed concentration, an increase of global LRV was observed with a stabilization around 2.5–3. This variation and the range of LRV obtained are in agreements with the results of Jacquet et al. (2021).



352

353 Figure 4: Variation of global LRV as the function of feed concentration for norovirus and
354 Tulane virus. LRV - Logarithmic Reduction Value ; C_r -Retentate concentration

355

356 The global LRV retention appears to be slightly higher in the case of norovirus compared to
357 Tulane virus (Figure 4). As mentioned above, the source of viruses (sewage sample or purified
358 cell culture supernatant) may explain the difference as organic matter generates membrane
359 fouling and can induce a higher retention. Indeed, it was shown that for water without natural
360 organic matter (NOM), the effect of electrostatic repulsions, ahead of size and hydrophobicity
361 effects, was modified by the NOM addition. It could both impact the aggregation of the NOM
362 with viruses and the membrane fouling with pore blockage, making size exclusion predominant
363 over other mechanisms (Cruz et al., 2017). Moreover, Jacangelo et al. (1995) showed that the
364 formation of a cake can increase retention by creating a second filtration barrier. For example,
365 the formation of a kaolinite cake on the membrane surface thus led to a higher retention of the
366 MS2 phage by a UF membrane from 1.2 to 3.7 log and the decrease of the permeate flux. In the
367 present work, the Tulane virus provides a good estimate of the retention of the norovirus by
368 ultrafiltration and consequently of its role as a surrogate.

369

370 In the case of average LRV, the best fit model was the Semi-log line (X is log – Y is linear),
371 corresponding to the equation type: Y=Yintercept + Slope*log(X) (Figure 5), without any
372 constraint given on the parameters for Tulane virus and norovirus.

373

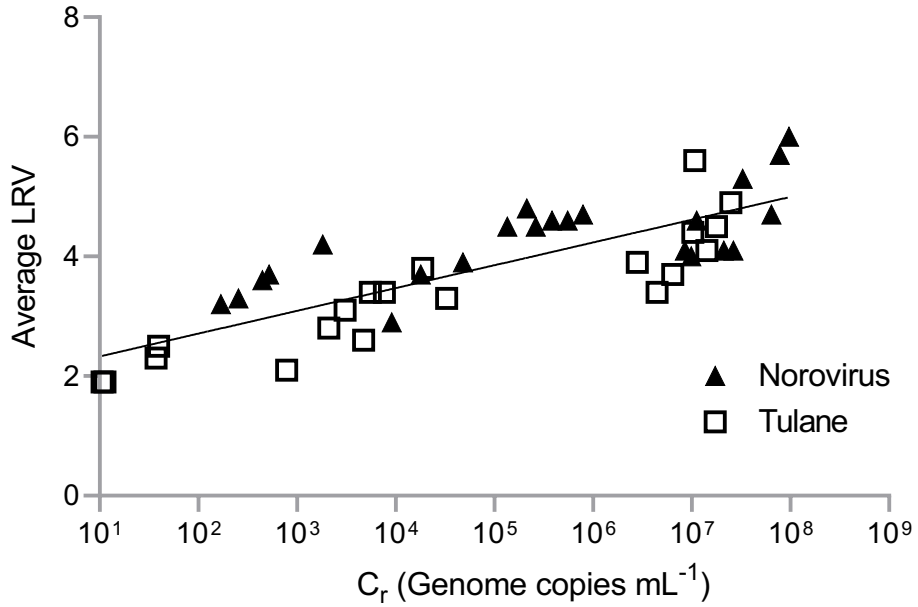


Figure 5: Variation of average LRV as the function of feed concentration for norovirus and Tulane virus. LRV - Logarithmic Reduction Value ; C_r -Retentate concentration

The presence of salts in seawater is an important factor that can influence viral retention by UF membranes, but this phenomenon remains complex to explain (Antony et al., 2012; Dishari et al., 2015; Gentile et al 2018; Huang et al., 2012; Wu et al., 2018).

For average LRV, for Tulane and norovirus and for the highest feed concentrations filtered, values higher than 2 were obtained. With the feed concentration, an increase of average LRV was observed to reach a value around 6 for an inlet concentration of 7.8×10^7 genome copies mL^{-1}. As for the average LRV, the retention based on global LRV of Tulane virus and norovirus are similar. The retention of norovirus seems slightly higher due to a more important fouling. Regarding these results, Tulane virus appears to be a good surrogate for norovirus in the case of its treatment by ultrafiltration.

3.4. Comparison of surrogate and virus retentions by UF

In the case of drinking water production, similar membranes are used to stop suspended matter, bacteria and viruses. Among the viruses and bacteriophages CV-B5 and MS2 bacteriophage are widely used (Jacquet et al., 2021). The size of CV-B5, 30 nm, is close to the size of norovirus and Tulane virus of 40 nm. The retention of Tulane virus and CV-B5 in fresh water (type Evian) was therefore compared. The choice of Evian water to make the comparison was based on results available in the literature (Taligrot et al. 2022; Jaquet et al. 2021). In fresh water, the

retention of Tulane virus and CV-B5 was identical whatever the upstream concentration, confirming the steric effect influence on the retention of viruses. Tulane virus appears as a good surrogate also for CV-B5 in the case of studies in drinking water production, but it is the MS2 bacteriophage which is often used to mimic the CV-B5 or ADV type 41 viruses. In fact, a large number of studies using enteric viruses and bacteriophages for the characterisation of viral retention of UF membranes have highlighted MS2 as a possible substitute for enteric viruses with similar retentions. The literature reports a low size exclusion contribution for experiments with similar pore and virus sizes (Shirasaki et al., 2017). With a low size exclusion contribution, virus removal by UF is then complex and depends on many mechanisms such as hydrophobic and electrostatic interactions (ElHadidy et al., 2013; Pontius et al., 2009) and/or virus aggregation (Jacquet et al., 2021).

Comparable results between Tulane and MS2 bacteriophage highlight that high feed concentration studies present a risk of overestimation of the UF performances with retentions around 3.0 log. In low feed concentrations (< 100 genome copies mL^{-1}), UF achieved retentions lower than 1.0 log for all viruses studied (ADV41, CV-B5 and MS2 phages). In the case of UF drinking water, the retention rates of the MS2 bacteriophage surrogate were similar or lower than for ADV41 and CV-B5, making the MS2 bacteriophage suitable as the "worst case" virus surrogate.

4. Conclusion

To validate membrane process for virus retention, surrogates offer advantages in terms of safety and ease of use. Tulane virus was not used yet to evaluate UF retention, regarding various experimental conditions, as virus concentration and type of water. For norovirus, the global LRV of less than 1 log were obtained for the lowest feed concentrations. Increasing initial feed concentrations results in increase of global LRV with a stabilization at 2.5 – 3 log. Similar results were obtained for Tulane virus in seawater, with a reduction rate up to 3 LRV, however global LRV retention appears slightly higher for norovirus. As explained in this paper, the presence of organic matter in the samples can generate a more important fouling leading to a higher norovirus retention. Homologous conclusions can be drawn from the average LRV comparison, where the values between 2 and 6 log (respectively for the lowest and the highest initial concentration) were obtained for the both viruses. Tulane virus prove to be a good surrogate for norovirus studies in a seawater.

The possibility of removing noroviruses from seawater by ultrafiltration offers a new solution for shellfish producers to improve sanitary quality of seawater used in the shellfish farm

installations (i) shellfish can be harvested and maintained in storage tanks supplied with pathogen-free seawater in case of contamination of production zone during norovirus risk period, (ii) to implement ultrafiltration in a shellfish depuration process by guaranteeing that the seawater used is free of viral particles and that the viral load excreted by the shellfish over time would be eliminated over time.

Acknowledgement

The project leading to this publication has received funding from FEAMP (APINOV N° FPEA470019FA1000001).

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