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Statistical approaches to understanding the impact of matrix composition on the disinfection of water by ultrafiltration

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HIGHLIGHTS

- Water matrix composition affects the disinfection efficiency by ultrafiltration.
- Bacterial removal was due to size exclusion and not affected by the presence of NOM.
- Viral removal in absence of NOM is governed by electrostatic repulsion theory.
- Aggregation of viral particles to humic acids enhanced their removal.
- Ion concentrations, ionic strength, and pH impacted the viral removal efficiency.

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ABSTRACT

We performed a systematic approach using statistical tools to understand the effect of the water chemistry on removal of microorganisms using ultrafiltration. We applied a four-factor at two-level factorial design with central point to synthesize forty mock solutions spiked with two pathogen surrogates, *Salmonella* Typhimurium and bacteriophage PP7, selected as bacterial and viral models, respectively. Calcium, magnesium, nitrate, and bicarbonate were the mono- and divalent ions considered as factors for the water matrix composition and their concentrations were based on actual ambient waters sourced for human consumption. The influence of natural organic matter (NOM) using commercial humic acids was also evaluated. The statistical analysis showed that steric exclusion was the main mechanism for bacterial removal independently of the presence of NOM. However, for the viral model in the absence of NOM rejection was governed by the electrostatic repulsion theory and the interaction of negative charged ions (nitrate and bicarbonate) played an important role. Aggregation of viral particles to humic acids enhanced their rejection, although removal efficiency was highly impacted by the interaction between chloride and calcium ions, ionic strength, and pH in the feed water. This approach can be applied in other membrane-based processes used in environmental engineered systems like wastewater treatments.

1. Introduction

Water scarcity and safe drinking water are two of the most serious global challenges of our time. In addressing these issues, the use of membrane technology has emerged as an invaluable tool in the water purification field [1]. Notably, low-pressure membrane filtrations such as microfiltration (MF) and ultrafiltration (UF) have gained widespread use for securing a safe water supply [2]. Numerous publications have shown that membrane processes can efficiently be used for drinking water disinfection [3,4]. In particular,

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UF compared to MF, is a process with a higher efficiency to remove particles, turbidity, large organic compounds, and microorganisms including pathogenic bacteria and viruses from contaminated feed streams [5,6]. Over the past two decades operational costs have been reduced significantly, as have membrane prices [2]. Furthermore, drinking water quality regulations have placed an increased emphasis on the removal of pathogens to ensure water safety [7,8] together with the removal of natural organic matter (NOM) as precursor for the formation of disinfection by products (DBPs). Therefore, membranes represent a suitable technology to reach the required disinfection level in drinking water, contributing to decreased public health risks for the exposed population.

Although the membrane pore size and the molecular weight cut-off (MWCO) are the main properties to consider to assess the rejection performance of filtration membranes employed in water treatment processes, these parameters alone are inadequate to estimate the pathogen removal efficiency [9]. The size exclusion mechanism is not enough to predict the expected separation due to several causes. First, pore size is frequently estimated by filtration of polymers like dextran or polyethylene glycol (PEG), which exhibit different structural and compositional properties than virus-like particles. Thus, the MWCO does not represent an uniform value but an average; therefore, the same values of MWCO from two different membranes do not necessarily mean that the pore sizes are homogenous, as most manufacturers measure them in different ways [5]. Indeed, abnormally larger pores, which are not included in the main pore size distribution, caused the leakage of virus during filtration [10]. Second, the removal efficiency can be highly influenced by the solution chemistry [11], changing the superficial characteristics of the membranes and the microorganisms, causing aggregation and/or repellence interactions.

Several studies have evaluated the influence of the water matrix composition on the UF performance based on quantity and quality of the filtrate. For example, some components studied were natural organic matter [12–15], pH, ionic strength, mono-, di- or trivalent salts [16,17] as single factors or combinations of them. For example, the interactions of NOM and divalent ions, ionic strength, and pH [11,18,19] have been reported. Furthermore, several authors studied the use of different polymer membranes like carbonate acetate, Polyethersulphone (PES), Polysulphone, and others [20,21], different configurations like hollow fibre and spiral wound [22], different pore size and modified membranes to enhanced UF performance [23–25]. Most of the reported studies have examined membrane fouling, flux reduction or rejection of a particular chemical element [14]. Others reported the effect of feed pre-treatments on UF performance with respect to NOM removal and flux reduction [26,27].

The removal of microorganisms, bacteria and/or viruses, along with one or more of the factors mentioned above has been addressed by only a few studies [10] even though the use of membranes for drinking water disinfection has been applied since the beginnings of the last century and the viral concentration has been reported since 1971 by Sorber and co-authors [3]. Viral removal by membrane systems and its relation to the feed composition has received increasing attention by several authors [10,17,28]. Although diverse membrane fouling mechanisms that influence viral retention have been proposed, the complexity of the water matrix composition needs further investigation. There has been a lack of a systematic approach, using factorial design and multivariate statistical tools, to understand the influence of mono-, and divalent salts, ionic strength, and organic material on the removal of bacteria and viruses by ultrafiltration at constant low pressure using PES membranes and sensitive molecular techniques for microbial detection. Quantitative real time PCR (qPCR) has been established as an appropriate technique to assess the use of membrane processes for the removal of viral particles from water [29].

This study was designed to analyse the influence of solution chemistry on the disinfection of drinking water. In order to investigate whether, or to what extent, humic acids and interactions with different salts influence the removal of pathogens, we used a factorial design with a central point approach to synthetize forty aqueous solutions. The concentration of ions and NOM utilised for the experimental design were the actual ones found in environmental waters sourced for human consumption in Salta, in the northwest of Argentina [30]. This region was selected due to its relevance and immediate need for an alternative treatment of the water consumed. Given the lack of a safe water distribution system, superficial and ground-waters exposed to several contaminants (i.e. poultry farms, latrines, among others) are the main sources of water consumed without treatment. This situation is similar in many developing and threshold countries where ultrafiltration for water disinfection (at the point of use POU or in pilot-scale drinking water plants) would represent a potential alternative to provide safe water for human consumption. Furthermore, in this region the concentration of NOM in natural waters is very low during winter (dry season) and extremely high during the wet season; therefore, it is relevant to study the effect of the NOM on the disinfection process.

To assess the pathogens removal, ultrafiltration was performed in a flat-sheet cross-flow unit and the forty synthetic solutions were spiked with surrogates of pathogens. *Salmonella enterica* subsp. *enterica* ser. Typhimurium and bacteriophage PP7 were used as bacterial and viral models, respectively. Assessment of the disinfection efficiency was performed using qPCR for the absolute quantification of the viral model and standard plate count methodology for the bacterial surrogate. Multivariate statistical analysis was used to evaluate effects of aqueous chemistry on the removal efficiency.

2. Materials and methods

2.1. Experimental design and synthetic aqueous matrices

The components and concentrations to synthesize the mock water matrices were selected based upon a year of monthly monitoring of two wells (shallow groundwater) and a river used as sources for human consumption [30]. According to their main constituents and actual concentrations, a factorial design to prepare the synthetic solutions was planned. The four main chemical components found, calcium (CA), magnesium (MG), nitrate (NI), and bicarbonate (BC), were chosen as the factors. Thus, the experimental design consisted of four factors at two levels (minimum and maximum concentrations) with the central point (average concentration) repeated four times (Tables 1 and 2).

Individual concentrated stock solutions containing per liter 5.8 g of $CaCl_2{\cdot}2H_2O$, 5.9 g of $MgCl_2{\cdot}6H_2O$, 3.0 g of $Ca(NO_3)_2{\cdot}4H_2O$, 3.0 g of $Mg(NO_3)_2{\cdot}6H_2O$, and 8.9 g of $NaHCO_3$ were prepared with sterilized Milli-Q water (NOM-free, deionized water additionally purified with granular activated carbon and a 0.2 μm filter; Millipore Water Purification System, Bedford, MA) and used to synthesize the mock matrices according to Table 2. Each ion concentration was measured following Standard Methods described by APHA [31].

Humic acids (HA) (Fluka-Sigma Aldrich, technical grade) were used as model of NOM. Twenty of the 40 solutions were synthetized without HA (corresponding to Block A) and the other twenty seeded with HA (3 mg/l final concentration) (corresponding to Block B). The concentration of 3 mg/l of humic acid was chosen based on the average concentration of NOM in natural waters monitored (3.33 mg/l, wet season) [30].

Table 1
Factors selected for the experimental design: calcium (CA), magnesium (MG), nitrate (NI), and bicarbonate (BC). The factors were evaluated at two levels (minimum and maximum). Additionally, a central point (CP) at average concentration for each factor was included.

Factors	Levels (mg/l)		
	Minimum (−1)	Maximum (+1)	CP
CA	10	50	30
NI	5	60	30
MA	5	15	10
BC	50	150	100

The humic acids stocks were prepared with Milli-Q water and stirred overnight for complete dissolution. The HA solution was then filtered using a 0.22 μm filter (CA, Millipore) and stored in the dark at 4 °C until use. The molecular weight (MW) distribution of the HA was determined by centrifugal fractionation of 15 ml of the 3 mg/l HA suspension, following the protocol described by [22]. Briefly, filters with nominal MWCO of 3, 10, 30, 50, and 100 kDa (Amicon, Millipore Corp., Bedford MA) were used and centrifugation was performed at 4000 rpm for 10 min for the 5, 30, and 50 kDa MWCO filters and 20 min for those with 3 and 10 kDa MWCO. Filtrates were collected and organic material was tested using the total organic carbon (TOC) analysis (TOC, SKALAR, Formacs TH).

The concentration of humic acids was measured by Absorbance at 254 nm (A_{254}, in cm^{-1}) using a spectrophotometer (Spectronic Unicam Genesys 10UV Model). This method was chosen because of its simplicity and the low concentration of HA (3 mg/l) used, since the dissolved organic carbon (*DOC*, in mg/l) method is less accurate for the low concentration ranges [14]. This was proven in the present work by measuring standard concentrations of HA in the range of 0–10 mg/l by both methods. The spectrophotometric technique had a higher correlation ($r^2 = 0.9998$) than the DOC method ($r^2 = 0.8408$). The specific UV absorbance (*SUVA*, in $l\ mg^{-1}\ cm^{-1}$) was calculated according to

$$SUVA = \frac{A_{254}}{DOC} \times 100 \quad (1)$$

The HA removal ($R_{HA,}$ %) was calculated using Eq. (2):

$$R_{HA}(\%) = \left(\frac{C_f - C_p}{C_f}\right) \times 100 = 1 - \left(\frac{C_p}{C_f}\right) \times 100 \quad (2)$$

where C_f and C_p are the HA concentrations in the feed (f) and permeate flow (p), respectively.

The water matrices with central point composition were repeated four times to estimate experimental standard deviation (Table 2). Each matrix was seeded with surrogates of virus and bacteria pathogens, and assessed randomly to avoid an outcome correlated to batch effects and leading to incorrect conclusions.

2.2. Surrogate suspension and detection system

Salmonella Typhimurium was used as the bacterial model while PP7, a bacteriophage of *Pseudomonas aeruginosa*, was used as the viral model. The standard plate count method with membrane filtration (24 h incubation time at 37 °C) was used to estimate the number of viable *Salmonella* in Salmonella-Shigella Agar (SS, Britannia, Argentina) and real-time Polymerase Chain Reaction (qPCR) was used for the absolute quantification of bacteriophage PP7 using a previously published oligonucleotide system [32].

Bacteria stock solutions were prepared from a culture in Luria-Bertani (LB) Broth (1.0% tryptone, 0.5% yeast extract, 1.0% sodium chloride, pH 7.0). *Salmonella* were incubated overnight in a flask containing 10 ml LB broth in a shaking incubator (Dalvo) at 37 °C. The biomass was separated from the broth by centrifugation at 4000*g* (Microcentrifuge, Eppendorf) for 10 min at 4 °C and the supernatant was discarded. Phosphate buffered saline (PBS) was added to the pellet, which was homogenized by vortex mixing. The suspension was centrifuged again to separate bacteria from PBS. This procedure was repeated three times to ensure the removal of all remaining nutrients to prevent further growth of bacteria. The final suspensions of *Salmonella* were added at a concentration of 10^9 CFU/ml (45 μl) into the solution composition of choice (4.5 L) to reach concentrations of 1.9–4.3 × 10^6 CFU/ml.

The bacteriophage PP7 (ATCC 15692-B2) was selected because of its small size (25–30 nm) and similar physicochemical properties to the poliovirus (the smallest member of the Enterovirus family, 27 nm); which represents the worst case scenario for the removal by ultrafiltration [32]. Furthermore, the bacteriophage PP7 has been chosen by the Parenteral Drug Association (PDA) as the viral model to test for small virus-retentive membrane-based filters [33]. While bacteriophage MS2 is the most widely used surrogate to characterize membrane viral removal, its suitability for this purpose has been questioned [9,29]. Stock solutions were prepared using an overnight culture of the host *Pseudomonas*

Table 2
Factorial design used to synthesize the mock solutions. Four factors were chosen: calcium (CA), nitrate (NI), magnesium (MG), and bicarbonate (BC). Two levels, maximum (indicated with 1) and minimum (indicated with −1) and a central point (CP, with four repetitions) were selected according to actual environmental concentrations (Table 1). Concentrations of ions chloride (CL) and sodium (NA) were calculated since those ions were included in the synthetic solutions as salts of added compounds.

Matrix	Factorial design				Ion concentration (mg/l)					
	CA	NI	MG	BC	CA	NI	MG	BC	CL	NA
M1	1	−1	−1	−1	50.0	5.1	5.0	50.1	100.2	18.9
M2	1	−1	−1	1	50.0	5.1	5.0	150.2	100.2	56.6
M3	1	−1	1	−1	50.0	5.1	15.0	50.1	129.4	18.9
M4	1	−1	1	1	50.0	5.1	15.0	150.2	129.4	56.6
M5	1	1	−1	−1	50.1	60.1	5.0	50.1	68.8	18.9
M6	1	1	−1	1	50.1	60.1	5.0	150.2	68.8	56.6
M7	1	1	1	−1	50.1	60.1	15.0	50.1	98.1	18.9
M8	1	1	1	1	50.1	60.1	15.0	150.2	98.1	56.6
M9	−1	−1	−1	−1	11.1	5.1	5.0	50.1	31.3	18.9
M10	−1	−1	−1	1	11.1	5.1	5.0	150.2	31.3	56.6
M11	−1	−1	1	−1	11.1	5.1	15.0	50.1	60.6	18.9
M12	−1	−1	1	1	11.1	5.1	15.0	150.2	60.6	56.6
M13	−1	1	−1	−1	11.2	60.1	5.0	50.1	0.0	18.9
M14	−1	1	−1	1	11.2	60.1	5.0	150.2	0.0	56.6
M15	−1	1	1	−1	11.2	60.1	15.0	50.1	29.2	18.9
M16	−1	1	1	1	11.2	60.1	15.0	150.2	29.2	56.6
M17-M20	CP	CP	CP	CP	30.6	30.0	10.0	100.1	66.1	37.7

aeruginosa (ATCC 15692) in nutrient broth, inoculated with PP7 and incubated overnight at 37 °C. In order to harvest viruses from bacteria debris, the virus suspension was centrifuged at 4000 rpm for 5 min, then the supernatant was filtered through a 0.22 μm sterile syringe filter (Millipore) to further remove debris. The filtered virus stock suspension was distributed into 100 μl-aliquots and stored at −80 °C until use. The concentration of PP7 in each resulting aliquot was approximately 10^{11} copies/ml.

2.3. RNA extraction, cDNA synthesis and qPCR protocol

Nucleic acids were extracted from 140 μl of each sample using the QIAamp Viral RNA kit (Qiagen, Valencia, CA) according to the manufacturer's directions, with a final elution volume of 80 μl, and stored at -80 °C immediately after extraction.

cDNA was synthetized using the Superscript III Reverse Transcriptase kit (Invitrogen) and adapted the manufacturer's instructions. Briefly, 10 μl of the nucleic acids extracted were added to 30 μl of the reaction mixture, giving final concentrations of: 1 × RT buffer, 500 μM dNTPs, 5 mM $MgCl_2$, 2 U/μl RNaseOUT, 10 U/μl SuperScript III, and 2.5 ng/μl of random hexamers. cDNA synthesis consisted of a 50 °C incubation step for 50 min, followed by incubation at 85 °C for 5 min to inactivate the RT enzyme.

Each 25 μl of qPCR reaction mixture contained 12.5 μl of commercially available 2X TaqMan® Universal PCR Master Mix (Applied Biosystems) with 0.5 μl of 50 μM primers (final concentration 900 nM) (Forward-GTTATGAACCAATGTGGCCGTTAT; Reverse-CGGGATGCCTCTGAAAAAAG) and 0.75 μl of 10 μM probe (final concentration 300 nM) (FAM-TCGGTGGTCAACGAGGAACTG GAAC-TAMRA), plus 5 μl of the cDNA sample, and water to complete the volume. Samples, negative and positive controls, all in duplicate, were placed in 96-well plates and amplified in an automated thermocycler 5700 ABI Instrument (Applied Biosystems) using a standard temperature profile (2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C). Results were analyzed with ABI software (Applied Biosystems). The C_t values (C_t: threshold cycle, number of cycle where the threshold fluorescence is reached) were calculated using as baseline cycles from 6 to 15 and a threshold of 0.03. The reaction efficiency was 97% (slope = −3.40). The potential inhibition of PCR due to the presence of humic acids was assessed using the dilution approach [32]. It was verified that 3 mg/l of HA did not inhibit the qPCR reaction.

2.4. Lab-scale cross-flow filtration system

Ultrafiltration experiments were carried out in a lab-scale flat-sheet tangential-flow filtration system, consisting of a custom-designed plate and frame cell module (Fig. 1). This type of membrane module was selected because it is easy to clean, sterilize and replace before and after each use to prevent cross contamination between experiments. The filtration unit consisted of: i) a 5 L feed tank containing the water matrix solution with microorganisms depending on the experimental requirements, ii) an UF rectangular cell (25 cm × 10 cm × 5 cm) with an effective membrane area (A) of 1.19×10^{-2} m^2, and iii) a peristaltic pump (APEMA, BS6D) with variable feed flow connected to the cell via a dampener to reduce pulsation and keep the pressure constant. The retentate was recirculated, while the permeate fluid was continuously discharged and weighed on an electronic scales (Shimadzu UX-2200H, Japan) serially linked to a computer for automated data collection at 1 min intervals. Tanks consisted of sterilized glass bottles and silicon tubes to assure low attachment of the microorganisms.

A commercial, flat, sheet-type Polyethersulphone (PES) ultrafiltration membrane (OT050, OMEGA, Pall Corp., USA) was used.

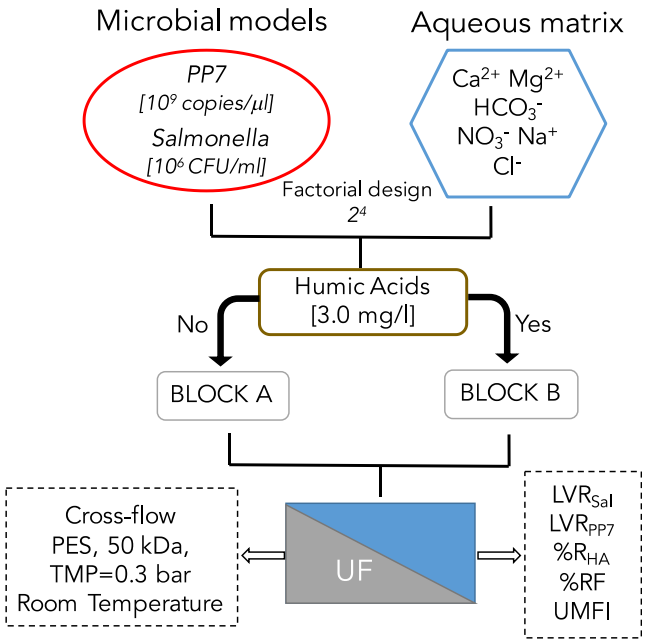

Fig. 1. Experimental set-up for the ultrafiltration of synthetic aqueous solutions according to a factorial design of four factors at two levels with four central points, without (Block A) and with (Block B) humic acids. A bacterial (*Salmonella*) and a viral (PP7) model were spiked into the aqueous solutions and their removal LVR_{Sal} and LVR_{PP7}, respectively, together with the removal of humic acids ($\%R_{HA}$) were calculated. The flux reduction ($\%RF$) and the unified membrane fouling index ($UMFI$) were also determined. TMP: Transmembrane pressure; PES: Polyethersulfone.

According to the manufacturer, the molecular weight cutoff (MWCO) was 50 kDa. Other characteristics of the membrane were: filtration flux 255.0 ± 17.0 ($l\,m^{-2}\,h^{-1}\,bar^{-1}$), nominal pore size (porometer) 66.8 nm and contact angle 68.9° ± 0.1° [25]. Each assay was performed with a new membrane, pre-treated by soaking overnight in distilled water to remove the hygroscopic compounds usually present on commercial membranes and sterilized following the manufacturer's instructions. First, compaction of each membrane was done for 3 h at 2 bar of constant pressure. Then, the hydraulic permeability, L_h ($l\,m^{-2}\,h^{-1}\,bar^{-1}$), was obtained through the filtration of sterilized Milli-Q water at different transmembrane pressure (TMP, ΔP) 0.3, 0.6, 0.9, and 1.2 bar. The hydraulic permeability was determined according to $L_h = J/\Delta P$, where J is the permeate flux ($l.m^{-2}.h^{-1}$) calculated as $J = V/(A \times t)$ (where t is the filtration time). The pure water flux (J_0) of the membrane was measured in the stabilized state. All the experiments were performed at room temperature (22–25 °C). The collected permeate volume (V, ml) was adjusted to 20 °C as the reference temperature, to account for the change of water viscosity, in order to obtain the volumetric flux (J).

2.5. Disinfection efficiency and fouling potential

Membrane performance was evaluated during disinfection by ultrafiltration of 4.5 L of water solution spiked with *Salmonella* (10^6 CFU/ml, final concentration) and PP7 bacteriophage (10^9 copies/μl, final concentration). The TMP was set to 0.3 bar and kept constant during the experiment run. Fluxes were normalized (divided by J_0) to compensate for the existing differences in the initial values and to compare between different assays. The bacterial and viral removal efficiencies were calculated in terms of the Log removal value (LRV) as:

$$LRV = -\mathrm{Log}\left(\frac{Cp}{Cf}\right) \quad (3)$$

The flux reduction (*FR*) as a typical fouling characteristic was determined by:

$$FR(\%) = \left(1 - \frac{J_p}{J_0}\right) \times 100 \quad (4)$$

where J_p is the flux measured at the end of the filtration.

To evaluate the level of fouling potential we used the unified membrane fouling index (UMFI) developed by Huang et al. (2008) defined based on the cake layer formation equation of Hermia's model, one of the most comprehensive for fouling prediction [34]. The proposed model defines *UMFI* (m^{-1}, or m^2/l) as:

$$\frac{1}{J_s'} = 1 + (UMFI)V_s \quad (5)$$

where J_s' is the normalized specific flux (dimensionless) and calculated according to:

$$J_s' = \frac{J_s}{J_{s0}} \quad (6)$$

and

$$J_s = \frac{J}{\Delta P} \quad (7)$$

(with J in m/s and ΔP in Pa in this case), and V_s is the specific volume (l/m^2).

The UMFI was determined by plotting the inverse normalized specific flux against the specific volume since J_s' and V_s were experimental data.

The UMFI is valid for both constant flux and constant, low-pressure membrane filtration thus, it allows a quantitative comparison between numerous membrane fouling results under different conditions regardless of membrane type, water source, operation mode and scale [34].

2.6. Effects of the selected factors on the response variables from the factorial design

Filtrations of forty different water solutions were performed. Twenty of these solutions were tested without humic acids (Block A) and the other twenty had a final concentration of 3 mg/ml of humic acids (Block B) and ion concentrations according to the experimental design mentioned above (Tables 1 and 2). The response variables measured were the removal of bacteria (LRV_{Sal}), viruses (LRV_{PP7}) and NOM (R_{HA}), as well as the fouling index (UMFI), according to the description provided in the previous sections.

To interpret the factorial design, the parameters were defined according to Eq. (8), where e is the error (calculated according to Eq. (9) with the CP data), C is the confidence interval and I is the variable influence and n is the number of experiments to estimate the error (four in the present study), t (t-Student, 95%) and Y_j is the response:

$$C = \frac{t \times e}{\sqrt{n}} \quad (8)$$

$$e = \sqrt{\frac{\sum_{j=1}^{n}(Y_j - Y)^2}{n-1}} \quad (9)$$

The principal effect (I_i) and the interactions between variables (I_{jk}) were determined according to

$$I_i = \frac{\sum_{j=1}^{2^V} X_{ij} Y_j}{2^{V-1}} \quad (10)$$

where subindexes i and k are the factors, j the experiment, V is the number of variables used (4), 2^V is the total number of experiments ran, $X = 1$ for the maximum level or $X = -1$ for the minimum level, and Y_j the response variable of experiment j. The influence of variable I on the response Y is significant only if $|I_i| > C$.

2.7. Multivariate statistical analysis. Principal components analysis

Data manipulation, merging of datasets and Principal Component Analysis (PCA), were performed with InfoStat software [35]. Multivariate statistical analysis was applied to assess and identify the impact of different factors on the efficiency of ultrafiltration to disinfect drinking waters. PCA was carried out to identify factors influencing the removal of bacteria and viruses by ultrafiltration. Only principal components (PCs) having eigenvalues greater than unity were considered of significant influence. PC loadings on the plots are interpreted as correlation coefficients between the variables and the factors, and represent how important a variable is for the obtained component. This interpretation is more reliable due to the fact that correlation eliminates errors caused by the different measurement scales for each kind of variable. Following the criteria of Liu and coworkers (2003) only loadings with strong correlation were considered as significant. The terms 'strong', 'moderate', and 'weak' refer to absolute loading values of >0.75, 0.5–0.75, and 0.3–0.5, respectively [36].

3. Results and discussion

3.1. Response variables from the ultrafiltration of synthetic solutions

The concentration of each component of the synthetic matrices was determined before and after each UF assay. The removal efficiencies and fouling potential were calculated as response variables (Table 3).

As there were two blocks of ultrafiltration experiments: Block A without HA and Block B with humic acids, the NOM molecular weight distribution was initially determined. For that, the aqueous solution of 3 mg/l commercial humic acids used as organic matter model in our experiments was divided into six fractions using cross-flow ultrafiltration technique (Fig. 2). Sixty-five percent of the HA fraction had a molecular weight larger than the MWCO of the PES membrane used (50 kDa) (Fig. 2) therefore, in theory, 65% was the presumed HA removal expected. Experimentally, the average HA removal (R_{HA}) was 62.1 ± 10.6% (Table 3) and the variability could be attributed to the different water matrix compositions discussed below.

The removal of *Salmonella* Typhimurium, used as the bacterial model, was higher than 4.9 log orders in all of the assays performed, with a removal average of 6.44 ± 1.22 LRV. When the measured concentration in the permeates was lower than the detection limit of the technique, a value of 1 CFU/100 ml was used for LRV calculations. Permeates with non-detectable values were expected since the bacteria size (a rod of 0.7–1.5 by 2.0–5.0 μm size) was larger than the membrane pore size 66.8 nm [25]. Jacangelo et al. (2006) also showed similar results during an inter-laboratory research study with different membranes and scales (bench and pilot plants) [6]. There was no statistically significant difference between the LRV_{Sal} with and without the presence of NOM.

Nevertheless, the viral model removal in Block B (with 3 mg/l HA) was significantly higher ($p = 0.00003$) than in Block A (without HA) (Fig. 3).

Theoretically, according to the following equation: $dp = 15.452 \times (MWCO \times 10^{-3})^{0.3371}$ [5], a PES membrane with 50 kDa MWCO (like the membrane used), should have a pore size of 0.1 nm, which is smaller than the viral particle used as model (27 nm). However, the removal in all the synthetic solutions was not completed, indicating that size exclusion played a partial role

Table 3
Response variables according to the factorial design used in Block A (without HA) and Block B (with 3 mg/l HA). LRV_{Sal}, $LRV_{Sal\text{-}HA}$: *Salmonella* removal without and with HA, respectively; LRV_{PP7}, $LRV_{PP7\text{-}HA}$: bacteriophage PP7 removal without and with HA, respectively; R_{HA}: humic acids removal; *FR*: flux reduction; *UMFI*: unified membrane fouling index (m^2/l).

Matrix	LRV_{Sal}	$LRV_{Sal\text{-}HA}$	LRV_{PP7}	$LRV_{PP7\text{-}HA}$	R_{HA} (%)	*FR* (%)	*UMFI* (10^{-3})
M1	6.42	6.98	1.53	2.73	67.46	25.84	1.40
M2	6.35	6.35	3.02	2.71	67.44	31.28	1.50
M3	5.86	6.10	1.72	3.17	59.00	21.18	1.10
M4	5.82	6.56	2.30	2.04	57.05	35.70	1.50
M5	5.40	7.23	3.44	3.31	43.06	18.00	0.80
M6	7.99	6.36	1.45	3.02	81.21	32.75	1.60
M7	8.40	6.59	2.77	2.99	68.39	28.72	1.20
M8	5.76	6.48	1.91	3.14	48.89	23.89	1.70
M9	5.74	6.51	1.86	2.95	55.89	44.57	2.70
M10	7.72	6.59	2.83	3.63	70.57	14.25	0.70
M11	4.89	5.39	1.50	2.33	74.17	29.00	1.00
M12	7.58	6.56	2.22	3.01	68.14	41.71	2.20
M13	5.87	6.67	1.91	3.47	72.85	29.90	1.10
M14	7.15	6.19	2.53	3.06	49.75	19.00	1.70
M15	6.23	7.86	2.53	2.70	54.43	29.26	1.30
M16	5.87	6.78	2.15	3.28	55.67	28.30	1.40
Average	**6.44**	**6.57**	**2.23**	**2.97**	**62.12**	**28.95**	**1.43**
SD	1.02	0.53	0.58	0.40	10.63	7.88	0.50
Min	4.89	5.39	1.45	2.04	43.06	14.25	0.70
Max	8.40	7.86	3.44	3.63	81.21	44.57	2.70
Central point (M17-M20)							
Average	7.0	6.5	1.9	2.6	56.7	27.7	1.60
SD	0.9	0.7	0.4	0.5	6.5	13.5	0.95

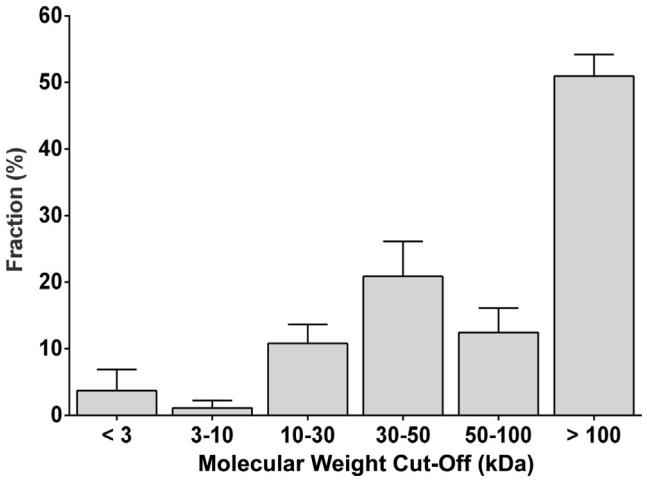

Fig. 2. Molecular weight distribution (MWCO) of humic acids (Fluka) used as Natural Organic Matter (NOM) in Block B assays.

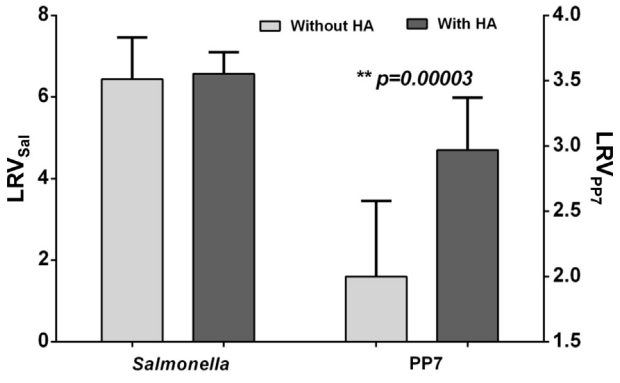

Fig. 3. Removal of the bacterial model *Salmonella* (LRV_{Sal}) and of the viral model PP7 (LRV_{PP7}), with and without added humic acids (HA) as a model of organic matter.

as a mechanism of removal/retention. Furthermore, it is clear that the presence of organic matter increased the removal of viral particles, which can be explained by aggregation and interactions between the virus and NOM [29], and also due to HA fouling [28].

3.2. Effects of the selected factors on the response variables from the factorial design

Statistical analysis of the factorial design showed that removal of humic acids is affected by the interactions of calcium (CA), bicarbonate (BC), and nitrate (NI), and also by the presence of all the factors considered for our experimental design (CA × MG × BC × NI) (Table 4). This result was expected since the pH and the ionic content of the solution have been widely reported as important factors in the removal of organic material by changing the surface properties of both the membrane and the NOM molecules [19]. However, our results showed that the interaction was negative, and therefore that higher concentration of ions decreased the removal of HA. We hypothesize that higher ionic strength, related to the higher concentration of ions, i) decreases the thickness of the double layer around the molecules and of the pore walls, and ii) decreases the charge repulsion of humic acid molecules, leading to a more coiled structure, thus reducing their removal [37]. Furthermore, when humic acids were not present, the bacteriophage removal was significantly affected by the interactions between NI and BC (Table 4, Fig. 4).

The removal of virus LRV_{PP7} decreased when the concentrations of ions BC and NI increased or decreased simultaneously (Fig. 4). Conversely, when the ions concentrations varied inversely, removal of the viral particles increased. However, this effect was not observed when organic matter was present in the aqueous matrices (Block B).

In order to compare and assess the effect of NOM in each water matrix, we represented the subtraction between $LRV_{PP7\text{-}HA}$ and LRV_{PP7} (Fig. 5). Virus removal is most affected by the presence of organic matter on matrices M6 and M13 where both exhibited higher values (>1.5 log). These two matrices had in common high concentration of NI and low concentration of MG; furthermore, they showed the highest HA removal, together with M11. These

Table 4
Principal effects and interactions of selected factors on the response variables: *Salmonella* removal without (LRV_{Sal}) and with HA ($LRV_{Sal\text{-}HA}$), PP7 removal without (LRV_{PP7}) and with HA ($LRV_{PP7\text{-}HA}$), humic acids removal (R_{HA}), flux reduction (FR), unified membrane fouling index ($UMFI$, m^2/l). Experimental error (e) and confidence interval (C) for central point. Four factors: calcium (CA), nitrate (NI), magnesium (MG), and bicarbonate (BC). Boldface values highlight statistical significant effects.

Effect/interaction	LRV_{Sal}	$LRV_{Sal\text{-}HA}$	LRV_{PP7}	$LRV_{PP7\text{-}HA}$	R_{HA} (%)	FR (%)	$UMFI$
CA	0.11739	0.01158	0.07434	−0.16551	−1.12008	−2.33031	−0.000163
NI	0.28549	0.39130	0.21300	0.30046	−5.68302	−4.21226	−0.000163
MG	−0.27801	−0.07088	−0.18672	−0.27726	−2.81024	2.77281	−0.000013
BC	0.67829	−0.18351	0.14425	0.03069	0.43402	0.05018	0.000213
CA × NI	0.48928	−0.22327	0.03687	0.15066	3.33442	1.55376	0.000113
CA × MG	0.19738	−0.22848	−0.00131	0.16916	−3.65130	−2.36385	0.000063
CA × BC	−0.71727	−0.10392	−0.33973	−0.35212	3.73290	7.41807	0.000238
NI × MG	0.24423	0.38603	0.19303	0.09021	−2.06312	−0.14044	0.000113
NI × BC	−0.45983	−0.45079	**−0.79151**	−0.02434	−1.23661	−0.53515	0.000288
MG × BC	−0.76570	0.29166	−0.12854	0.04032	−6.99234	5.30959	0.000338
CA × NI × MG	0.22293	−0.34751	−0.11291	−0.08180	5.02591	0.66583	0.000087
CA × NI × BC	0.47422	0.24916	−0.43310	0.27740	6.39163	−1.97630	−0.000088
CA × MG × BC	−0.53475	0.16868	0.18343	−0.20755	**−7.90442**	−7.93425	−0.000338
NI × MG × BC	−0.95176	−0.25433	0.16121	0.31562	−1.33292	−7.71883	−0.000538
CA × NI × MG × BC	−0.36458	0.16948	0.34561	0.06600	**−12.59756**	0.55454	0.000388
e	0.92527	0.68162	0.37659	0.49941	6.47406	13.57519	0.000949
C	1.08719	0.80090	0.44249	0.58680	7.60702	15.95085	0.001115

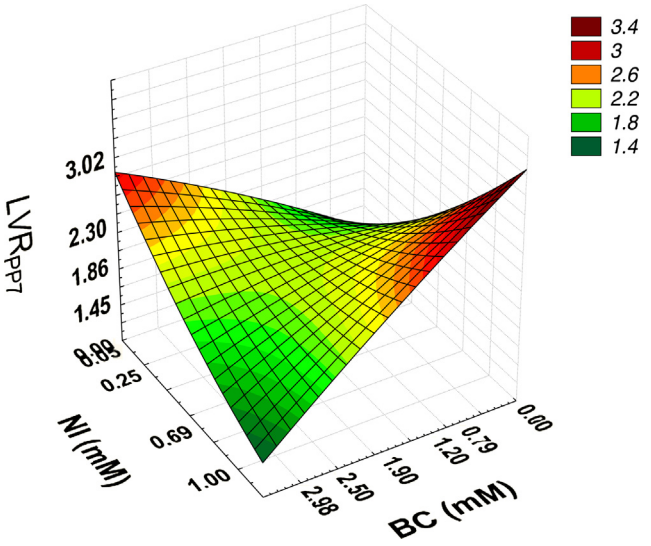

Fig. 4. 3D surface plot (adjusted to a quadratic function) of the PP7 removal (LRV_{PP7}) and nitrate (NI) and bicarbonate (BC) interactions without humic acids.

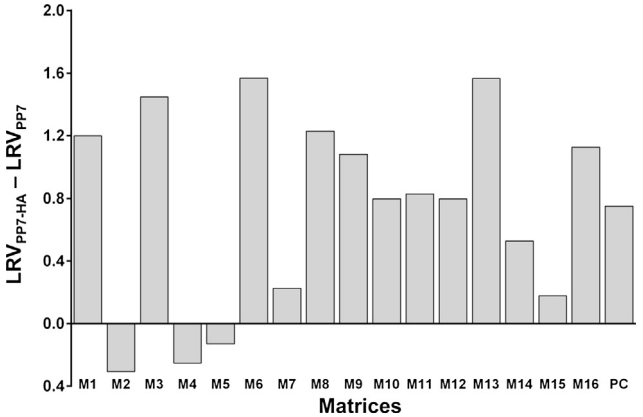

Fig. 5. Effect of humic acids on PP7 removal: with ($LRV_{PP7\text{-}HA}$) and without (LRV_{PP7}) humic acids, for each water matrix.

results suggest that the removal of viral particles was enhanced due to their aggregation to the organic matter substances.

The lower virus removal showed by the matrices without the addition of organic matter might indicate that not only the size exclusion mechanism but also electrostatic/repulsion forces took place during ultrafiltration assays. Particles exhibiting the same charge as the membrane surface are generally less likely to cross through the membrane, as they are repelled back to the bulk feed solution by charge repulsion, thus their removal is also enhanced. Also, Farahbakhsh (2004) suggested that initially coliphage removal is due to adsorption of the viral particles to the membrane [38], but this situation changes with the accumulation of different ion charges on the membrane. According to Zularisam et al. (2007), based on the DOC removal of different NOM fractions by hydrophobic and negatively charged membranes like polysulphone or PES, the rejection mechanism decreases: electrostatic interaction > hydrophobicity > steric exclusion; meanwhile in less negative and hydrophilic membranes (i.e. cellulose acetate) the order is changed: steric exclusion > electrostatic interactions > hydrophobicity [39].

Nap et al. (2014) demonstrated that the overall surface charge density of the bacteriophage PP7 capsid is a complicated balance with the chemical dissociation equilibrium of the amino acids (capsid proteins) and the electrostatic interaction between them, and the translational entropy of the mobile solutions ions [40]. However, when the ionic strength increased the zeta potential of PP7 turned less negative, which could decrease phage removal since they are prone to pass through the membrane. Two characteristic functional groups are present in the PES structure, one is hydrophilic (sulfonil group) and the other hydrophobic (benzene ring). The metallic ions with positive charges interact, completely or partially, with the negative atomic fractions of the membrane; the most probable interaction sites are the two O-atoms ligated to S in the sulfonil group [41]. Thus, the membrane could be neutralised and the addition of cations (the bicarbonate added was $NaHCO_3$) might increase the attraction of viral particles to the membrane because the Stern potential decreased. Therefore, motion through the pores was facilitated and viral removal was decreased.

To confirm that there was repulsion or adsorption between the viral particles and the membrane, we performed ultrafiltration experiments with milliQ water without any other salts (no ionic charges) and at neutral pH. The viral removal was 2.6 log, higher than many of the Block A assays. This showed that electrostatic

repulsion, due to similar charge of the bacteriophage and the membrane, increased the removal in our system. Nap et al. (2014) demonstrated that at approximately pH 6–7 (used in the present work) the outer surface of the PP7 capsid is already negatively charged since it is composed principally of the amino acids aspartic and glutamic acid [40].

The presence of organic matter enhanced the viral removal significantly ($p < 0.01$). Two mechanisms are responsible for the improved viral removal: i) the partial blockage of pores by HA and ii) the aggregation and interactions between HA and viral particles. Both processes enhanced the rejection power of the membrane. It is interesting to note that the presence of a high concentration of calcium (in matrices M2, M4, M5, and M7, Fig. 5) with HA, did not enhance the virus removal, similarly to what was obtained by Huang et al. (2012) [17], but contrary to what we initially expected according to other studies [6,38] although the membranes used there had larger pore sizes. Our hypothesis is that in those matrices Ca^{2+}, together with humic acids, created a denser cake-formation and enhanced concentration polarization. Simultaneously, the increased IS given by ions Ca^{2+} reduced or supressed electrostatic repulsion between the viral particles and the membrane (both negative at the working pH), and allowed their passage through the membrane pores, decreasing the viral removal.

Contrary to what was reported by many authors [11,39] HA removal was greater when the factor (ions) concentration was lower. This led us to hypothesize that higher ion concentrations built a stronger cake formation therefore enhancing concentration polarization and accumulation of HA in the pores leading to an enriched organic matter transport across the membrane [28].

3.3. Multivariate analysis

Principal Components Analysis (PCA) was performed in order to assess all the factors not considered initially but with enough data recorded to perform the analysis. Therefore, besides the four factors of the original factorial design (CA, NI, BC, and MG) and the response variables (LRV_{Sal}, LRV_{PP7}, *RF*, R_{HA}, *UMFI*) we also considered ionic strength (*IS*), concentrations of sodium (NA) and chloride (CL). After several analyses with all the factors and response variables, we selected those that showed a significant effect. For further analysis, in Block A we included ionic strength (*IS*). In Block B chloride concentration (CL), humic acid removal (R_{HA}), flux reduction (*FR*), and *UMFI* were taken into account. Blocks A and B were analyzed separately, including the corresponding central point (CP) assays for each case.

3.3.1. PCA for Block A (without humic acids)

For the PCA analysis the ionic strength was calculated considering all the ions present in the solutions, including sodium and chloride, together with the ions of interest (CA, MG, NI, and BC) during the preparation of the synthetic solutions. The two monovalent ions were in high concentrations in our water matrices (Table 2) and their influence on viral removal was reported to be important by others authors [9] (Fig. 6).

The PCA analysis identified three components (PC1, PC2, and PC3) accounting for more than 55% of the total variability. The PC1 explained 36% of the variability, and it was strongly related to ions CA, BC, and to the *IS*. Thus, we define PC1 as a mineral component that showed a positive impact on bacteria removal. Nineteen per cent of the variability was explained by the PC2, and showed a very interesting, moderate, inverse interaction between PP7 and magnesium. According to Brady-Estevez et al. (2010) the decrease on the viral removal efficiency is because Mg^{2+} cations are highly hydrated in the aqueous solution, and thus develop repulsive hydration forces not allowing the adhesion or attachment of the microorganisms to the membrane and their aggregation is lower [42]. Therefore, their hydrodynamic ratio decreased and consequently their removal also decreased. The PC3 also showed an inverse interplay between ions magnesium and the removal of the bacterial surrogate and, in this case, enhanced by the strong presence of nitrate.

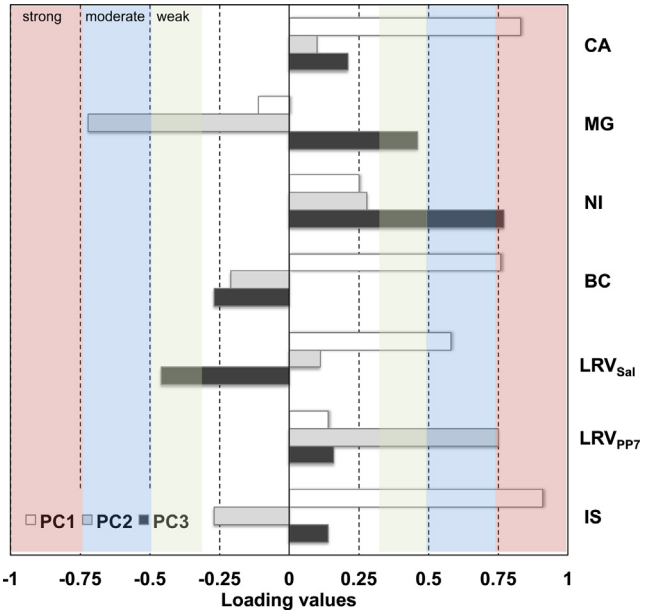

Fig. 6. Principal Component Analysis for Block A (without humic acids). Three main components were analyzed (PC1, PC2, and PC3) for the concentration of calcium (CA), magnesium (MG), nitrate (NI), bicarbonate (BC) ions, the ionic strength (*IS*) and the removal of the bacterial (LRV_{Sal}) and viral (LRV_{PP7}) models. The terms 'strong', 'moderate', and 'weak' refer to absolute loading values of >0.75, 0.5–0.75, and 0.3–0.5, respectively [36].

3.3.2. PCA for Block B

For Block B we included the chloride concentrations (CL) that were provided by salts used to prepare the synthetic solutions (Fig. 7).

The PCA analyses identified four principal components accounting for more than 65% of the total variability. The PC1 explained 27% of the variability among the matrices in Block B. Considering strong and moderate loading values, this PC related positively to CL, CA, BC, and MG and inversely to PP7 removal. Calcium and magnesium ions favour the aggregation of the viral particles when humic acids are present thus, the fouling increased and therefore, viral particles removal improved. However, other researchers reported that after a certain calcium concentration is reached those effects were not observable [43]. Other authors showed a decrease in the disinfection efficiency when NOM and calcium were added using carbon nanotubes.

The decrease of virus removal with the increased concentration of chloride (CL) was enhanced with the decline of calcium ion concentrations (Fig. 8). The binding capacity of the divalent ions (calcium and magnesium) with the NOM, which leads to flux reduction during filtration and generally improves the selectivity, is widely known. However, in these matrices the chloride concentration is high enough to mask the binding effect of the divalent cations (magnesium and calcium salts provide the chloride ions). This effect can be explained from the electrostatic repulsion forces theory, taking into account that we had Na^{+}, Ca^{2+}, and Mg^{2+} as cations and NO_3^-, HCO_3^-, and Cl^- as anions at different concentrations in the synthetic solutions. Sutton and Sposito [44], showed the higher interaction of NOM with calcium ion due to strong binding between the carboxyl groups of the NOM. Meanwhile, another

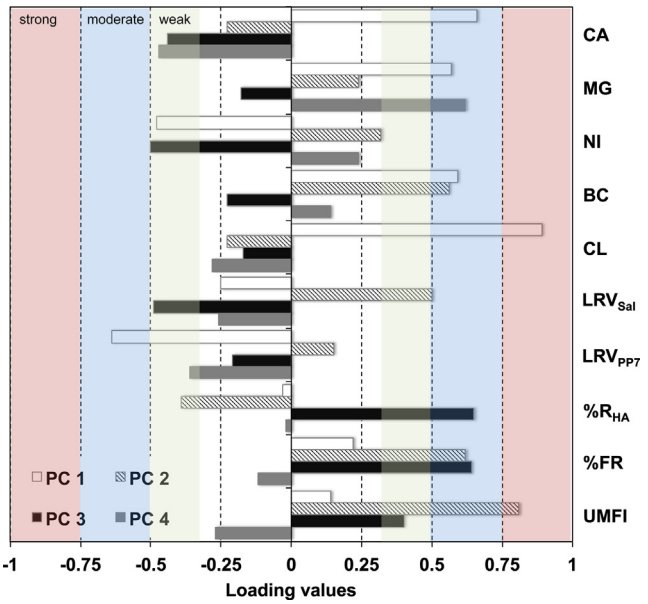

Fig. 7. Principal Component Analysis for Block B (with 3 mg/l humic acids). The terms 'strong', 'moderate', and 'weak' refer to absolute loading values of >0.75, 0.5–0.75, and 0.3–0.5, respectively (Liu, 2003).

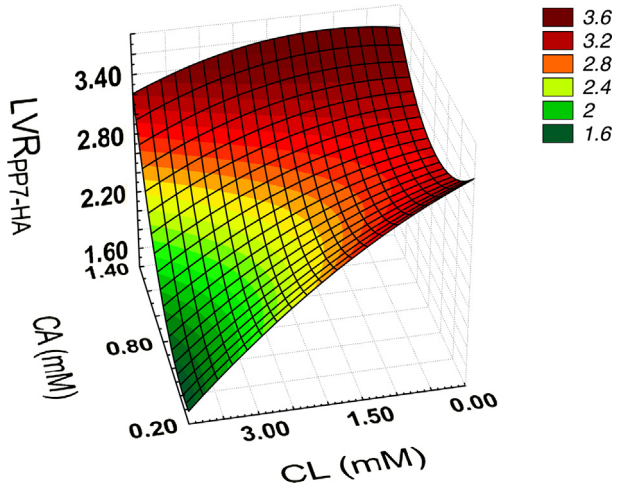

Fig. 8. Interactions between calcium (CA) and chloride (CL) ions and PP7 removal ($LRV_{PP7\text{-}HA}$) with 3 mg/l humic acid (HA) added.

study performed by Ahn et al. (2008) using molecular models demonstrated that the Na^+ quickly moves toward the bulk solution rather than binding the NOM or the sulfonil groups of the PES due to its small positive charge [41]. Simultaneously, as the PES membrane is in contact with water, hydrogen bonds with the sulfonil groups are formed on its surface, producing as a consequence a less negatively charged membrane. Furthermore, in the bulk solution there are Na^+ cations and most of the anions, principally Cl^-, which create very strong repulsive electrostatic forces not allowing the viral particles to aggregate. Therefore, the particles preserve their small hydrodynamic ratio and thus their passage across the membrane is more likely.

The PC2 explained 20% of the variability and showed positive correlation between flux reduction and *UMFI*, thus this PC2 is related to fouling formation, which also increases bacterial removal. That relationship was highly expected because the flux and specific volume are used to calculate the fouling index. PC2 also showed moderate and weak loading values for factors BC and R_{HA}, respectively, which are inversely correlated. This could be explained based on the pH and the configuration of HA discussed below.

The PC3 explained 18% of the variability and showed a strong direct relation with HA removal and flux reduction, but not with *UMFI*. The average HA removal was around 62%, as expected accordingly to the molecular size distribution (Fig. 2). However, there were cases with removal values lower than presumed which could be related to the pH of the matrix synthesised. The pH was measured in each matrix but not modified, because it was within the actual range (pH = 7.0–8.7) found in the ambient waters studied [30]. Indeed, those few solutions where the pH was slightly higher (pH = 8.5) showed lower HA removal. This finding is in agreement with Al-Amoudi (2010) who reported that the fouling (retention) decreases when pH increases, therefore the flux is higher or does not show a decline [16].

Many authors agree that the primary component responsible for fouling is the organic matter and within this, humic acids are the most important [12,14]. The presence of divalent ions causes more severe fouling by NOM [11]. Jermann et al. (2008) demonstrated that the formation of a humic acid layer on the membrane is increased by the presence of calcium ions [15]. The mechanisms involved are aggregation of HA and divalent ions acting as "chelating" bridges between the negative charged membrane surface and negative humic acid species. However, this effect was not detected in our study at the concentrations tested. On one hand, it could be because of the preference of ions for the NOM rather than for the PES. On the other hand, it could be due to the HA composition. A value of *SUVA* < 2.0 indicates that hydrophilic elements are the main components (low hydrophobicity) of the humic acids and low molecular weight are the predominant; meanwhile *SUVA* > 4.0 indicates higher hydrophobicity with carboxyl and phenol fractions [45]. The determined *SUVA* of 2.9 L cm^{-1} mg^{-1} of the humic acids used as our NOM model indicated a slightly higher presence of hydrophilic materials, therefore less hydrophobicity and therefore less likely to be the cause of irreversible fouling. However, the 35% of the HA fraction that was smaller than 50 kDa could cause cake-formation, which is of considerable importance since hydrophobicity is a secondary factor when the organic matter size can promote irreversible fouling.

4. Conclusions

The use of synthetic feed waters with controlled composition in terms of ions, organic matter, and pathogen surrogates allowed us to understand at a fundamental mechanistic level how the interactions between the solution components influenced the removal processes performance beyond the membrane pore size or the steric exclusion phenomenon. We conclude that in the absence of NOM, the viral particle repulsion (more rejection) is governed by the electrostatic repulsion theory, rather than steric or hydrophobic interactions, and the interaction of negative charged ions (i.e. nitrate and bicarbonate) plays an important role. This finding is important for regions where the concentration of NOM in natural waters is very low or when other pre-treatment technologies are used to remove NOM from the feed. On the other hand, when NOM is present it is proposed that aggregation of the viral particles, cake formation and pore blockage are the fouling mechanisms operating to enhance virus removal. Even though the flux reduction was related to higher removal of humic acids, there was no association with the removal of viral particles. Indeed, the interaction between ions chloride, calcium, and NOM increase or reduce the viral removal, according to the *IS* and the pH in the feed water. The statistical tools applied can be useful for future modelling analyses including virus, NOM, and solution chemistry of environ-

mental engineered membrane-based processes, and suitable for the design of drinking water and wastewater treatment plants considering the seasonal variation of the water quality sourced.

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