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Direct ultrafiltration performance and membrane integrity monitoring by microbiological analysis

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ABSTRACT

The feasibility of substituting a conventional pre-treatment, consisting of dioxi-chlorination, coagulation/flocculation, settling and sand filtration, of a drinking water treatment plant (DWTP) by direct ultrafiltration (UF) has been assessed from a microbiological standpoint. Bacterial indicators, viral indicators and human viruses have been monitored in raw river, ultrafiltered and conventionally pre-treated water samples during two years. Direct UF has proven to remove bacterial indicators quite efficiently and to a greater extent than the conventional process does. Nevertheless, the removal of small viruses such as some small bacteriophages and human viruses (e.g. enteroviruses and noroviruses) is lower than the current conventional pre-treatment.

Membrane integrity has been assessed during two years by means of tailored tests based on bacteriophages with different properties (MS-2, GA and PDR-1) and bacterial spores (*Bacillus* spores). Membrane integrity has not been compromised despite the challenging conditions faced by directly treating raw river water. Bacteriophage PDR-1 appears as a suitable microbe to test membrane integrity, as its size is slightly larger than the considered membrane pore size. However, its implementation at full scale plant is still challenging due to difficulties in obtaining enough phages for its seeding.

1. Introduction

The limited fraction of water suitable for human consumption together with the increasing population and water demand leads to the need of using alternative water resources, sometimes of compromised quality. Among the factors related to the use of challenging water resources, over a hundred of different pathogenic waterborne organisms including protozoa, bacteria and viruses have been identified as a topic of concern for drinking water production (Hunter, 1997). As a result, the use of advanced technologies, among them membrane filtration processes, has gained increasing attention. In particular, low pressure membrane systems have experienced an increase, almost exponential, in globally installed volume (Gimbel, 2003), with 60% of its applications in the drinking water treatment sector (David et al., 2008).

Low pressure membranes (microfiltration (MF) and ultrafiltration (UF)) retain suspended solids and microbes greater than their pore size by size exclusion mechanisms. Bacteria and protozoa are in general highly retained by membrane processes (Jacangelo et al., 1997). Loose of membrane integrity could enable the passage of those microorganisms or compounds greater than its nominal pore size, resulting in compromised water quality. Several reasons can cause the failure of membrane fibers (Guo et al., 2010): chemical corrosion, incorrect installation and maintenance, inappropriate operation conditions causing stress and strain and impairment by objects not removed in the pre-treatment. Nevertheless, membrane operation can also potentially increase membrane performance: membranes' pore clogging could result in an apparent tighter pores size distribution, enhancing the removal by sieving effect.

Besides size exclusion, adsorption and electrostatic interactions can also lead to a certain rejection of compounds and microbes smaller than the pore size. In the case of UF only some viruses are minor than the pore size of the membranes (EPA, 2003). Adsorption of viruses smaller than the membrane pore size depends on the

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nature of the membranes (Sobsey and Glass, 1984; Herath et al., 1999; von Voorthnizen et al., 2001), the water characteristics such as ionic strength, organic matter content, etc. (Sobsey and Glass, 1984; Herath et al., 1999; Langlet et al., 2009), the coating of the membrane with adsorbed, mostly organic, matter or the biofilm formation (Ueda and Horan, 2000; Farahbakhsh and Smith, 2004; Marti et al., 2011) and the viruses themselves (Langlet et al., 2008, 2009; Michen and Graule, 2010; Goodridge et al., 2004). Therefore, regarding virus retention, all these factors entail low pressure membranes becoming a barrier with potentially changing efficiency, even in the case that the membrane remains intact and the quality of water to be filtered is kept constant.

The efficiency of a membrane regarding the retention of viruses can be assessed either with the human viruses themselves or with bacteriophages used as surrogate viral indicators (IAWPRC Study Group, 1991; Jofre, 2007; Lucena and Jofre, 2010). Human viruses are relatively scarce in potential water resources and their seeding in experiments is not recommended for sanitary reasons and it is hardly feasible when great volumes of water are tested (e.g. in pilot/full scale installations). On the other hand, naturally occurring bacteriophages used as indicators of human viruses, such as somatic coliphages and F-specific RNA bacteriophages (IAWPRC Study Group, 1991) can be used to monitor the performance of a given membrane system (USEPA, 2001; IAWPRC Study Group, 1991). Due to the characteristics of certain bacteriophages (different sizes, different surface properties, easiness to grow large concentrations in the laboratory) they appear as appropriate to conduct membrane integrity tests. As an example, MS-2, sized 20–25 nm, is recommended by USEPA for the assessment of integrity of reverse osmosis (RO) membranes (NSF-EPA, 2011).

Alternative drinking water treatment schemes need to demonstrate their physico-chemical and microbiological removal capacity. Direct UF has proven its capacity to substitute DWTPs conventional pre-treatment from a physico-chemical and an operational standpoint, using full scale modules and continuously dealing with challenging surface water (Ferrer et al., 2013a; Galvañ et al., 2014, Briceño et al., 2014). An advantage of this configuration is its microbiological removal capacity which does not require chemical disinfectants. Nevertheless, up to the moment it has not been fully characterized using bacteria, bacteriophages and human viruses simultaneously. Taking into account that direct UF relies solely in a single membrane filtration step and that it would replace an initial disinfection and coagulation/flocculation and settling, which are the stages that remove microbes to a significant extent within a DWTP (WHO, 2004), it becomes necessary to study in detail its long term removal capacity to protect public health from microbial risk. Hence, this work compares the performance of direct UF with conventional pre-treatment from a microbiological standpoint, analyzing traditional bacterial indicators (*Escherichia coli* and spores of sulfite reducing clostridia (SSRC)), viral indicators (somatic coliphages and F-specific RNA bacteriophages) and human viruses (culturable enteroviruses measured as plaque forming units (PFUs) on BGM cells, and genome copies (GC) of enteroviruses (22–25 nm) and genotypes I and II of noroviruses (27–38 nm)) during two years.

Membrane integrity is of utmost importance to guarantee its removal capacity, especially in the treatment scheme considered here (direct UF). The best process indicator in terms of virus removal currently is challenge testing with MS-2 bacteriophage (Antony et al., 2012). However, other surrogates for virus removal such as Qβ are also being used (Matsui et al., 2003; Matsushita et al., 2005; Otaki et al., 1998; Shirasaki et al., 2007; Urase et al., 1996), but at bench scale. Langlet et al. (2009) quantified lower removal rates of Qβ than MS-2 and suggested it as a better candidate to determine membrane virus removal in worst case scenarios. Taking into account that virus rejection depends on several parameters related to the membrane and the feed water characteristics as well as the microbe itself, there is a need to determine the suitability of different organisms for microbes integrity testing as well as to validate it with full scale modules over a long period of time with real water. Indeed, there is limited information on ageing effect on pathogen removal (Antony et al., 2012). This work has compared bacteriophages MS-2 (22 nm) and GA (22 nm), both smaller than the membrane nominal pore size (40 nm) but differing in surface charge and hydrophobicity, PDR-1 (60–70 nm) bacteriophage, slightly greater than the pores, and *Bacillus* sp. spores (length > 1000 nm; diameter > 500 nm) much greater than the membrane pores. As a result, surrogates with different properties and hence, potentially different behavior, have been considered, providing information on membrane integrity and virus removal. Additionally, their removal has been compared to physico-chemical indicators (turbidity and UV_{254}) to determine if those parameters, easier to measure, could provide equivalent information. Finally, these tests have been conducted during two years to monitor membrane integrity of the direct UF membrane, to identify whereas changes occurred due to fouling or ageing. Long term studies (2 years) at pilot scale dealing with real water, assessing both naturally occurring and spiked microorganisms, with low detection limits, if existing, are scarce.

2. Materials and methods

2.1. Case study and experimental set up

The case study selected was Sant Joan Despí Drinking Water Treatment Plant (SJD DWTP), located in the vicinities of Barcelona (Spain), which treats Llobregat River water to provide drinking water almost to 50% of the population of the Metropolitan Area of Barcelona, equivalent to approximately 1.5 million inhabitants (CETaqua and Fife-Schaw, 2010). The Llobregat River presents a typical Mediterranean character, representing a challenging water source due to its high variability in terms of water quality and quantity. The SJD DWTP is a complex multistep treatment that combines a conventional pre-treatment, consisting of an oxidation with chlorine dioxide, coagulation–flocculation, settling, sand filtration, followed by either ozonation and activated carbon filtration or by UF and RO with a final post chlorination step. In this study, the results of a prototype plant consisting on direct UF of Llobregat River raw water has been compared to the results achieved by the current initial steps of SJD DWTP in terms of microbiological content removal, to assess its capacity to substitute the existing conventional pre-treatment (dioxi-chlorination, coagulation/flocculation (with aluminum salts), settling and sand filtration).

The direct UF prototype, of 15 m^3/h nominal capacity, consisted of a strainer (1 mm) and a coagulation tank with a stirrer, used when coagulation with ferric chloride was needed, submerged outside-in polyvinylidene difluoride (PVDF) membranes (1 membrane cassette with 10 Zeeweed® 500D modules) contained in a 8,000 L feed tank, pumping systems, reservoirs, dosing and sampling points, as well as an automatic control and data acquisition system. The nominal pore size of the membranes installed was 40 nm and 0.8 mm and 1.99 mm the internal and external fibres diameter, respectively. The prototype operated continuously since May 2011 until July 2013 facing very different scenarios of Llobregat River water quality (e.g. turbidity ranging from 8 up to >1000 NTU). Further details of the prototype and its operation optimization can be found in Galvañ et al. (2014).

The sampling campaigns were conducted simultaneously in the direct UF prototype and in the conventional pre-treatment in order

to have comparable results. Membrane integrity tests were only performed in the direct UF prototype. Sampling points were raw river water (Llobregat River), prototype permeate and sand filtered water (Fig. 1). The sampling frequency depended on the test: bacterial indicators analyses once or twice a month and virus analyses and membrane integrity tests every 3–4 months, during two years.

2.2. Membrane integrity tests seeding stocks preparation and tests performance

Highly concentrated stocks of phages MS-2 and GA were obtained on host strain *Salmonella thyphimurium* WG49 according to ISO (1995) and stored at −80 °C in 20% glycerol until use.

PRD-1 stocks were obtained on *E. coli* MS1000. For such purpose, PRD-1 phages were plated with 3 mL of top agar (ISO, 2000) and 0.2 mL of an *E. coli* MS1000 overnight culture and were incubated at 37 °C. After 24 h, the phage lysate was re-suspended in 4 mL of peptone saline solution (ISO, 1995) and centrifuged at 3000 rpm for 10 min. The supernatant was filtered through a 0.22 μm pore size low protein binding polyether sulfone membrane filter (Millipore, Bedford, MA) and stored at −80 °C in 20% glycerol until use.

Bacillus sp spores were obtained by feed-batch fermentation in a 10 L bioreactor with glucose and acid hydrolyzed casein as the main nutrients. The culture was left to grow and to produce spores during 72 h. Spore making was determined by phase contrast microscopy. Then the culture was harvested and centrifuged in a GEA Westfalia Separator disc stack centrifuge up to 8000 × *g* at 20 °C. The cell/spore concentrated were centrifuged, pelleted and washed three times in Ringer solution at 12,000 × *g* at 4 °C. The final pellet was re-suspended in a final volume of 1200 mL and distributed in containers holding 50 mL, which were treated at 80 ± 1 °C during 60 min to eliminate vegetative cells. The spore content of the suspensions ranged from 1.6×10^9 to 5.3×10^{10} spores per mL and diluted to contain about 10^{10} spores per mL, since greater concentrations generated a lot of aggregation among spores. The suspensions were kept at 4 °C until use.

The protocol used to conduct the membrane integrity test was the one described in Ferrer et al. (2013b). Initially, a chemically enhanced backwash (CEB), under the conditions recommended by the membrane manufacturer, was conducted and afterwards the feed tank was drained and manually cleaned. Then, it was filled with 8,000 L approximately of drinking water and it was conditioned with sodium thiosulfate (JT. Baker, 1N, Analyzed Grade) to remove any potential free chlorine presence, and sodium chloride (JT. Baker, ACS grade) to achieve 2000 μS/cm, to keep constant the ionic strength of the solution. The prototype filtered at 24.5 L/($m^2 \cdot h$) (LMH) during 2 min to flush the pipes and to measure the membrane permeability before the test (to quantify the extent of membrane fouling not removable by chemical means). Subsequently, the test microorganisms were spiked into the membranes' tank by pairs in the following way: one with phages MS-2 and PDR-1 and the other with GA phage and *Bacillus* spores. For each indicator, 1 mL of a high title suspension (10^{10} or greater indicator per mL, aiming at ensuring between 10^3 and 10^4 Plaque Forming Units (PFUs) or Colony Forming Units (CFUs) per mL in the feed tank) was diluted in 1 L of feed water. After thorough agitation, a 5 mL aliquot was taken to count the indicators seeded and the rest was poured to the membrane's tank. Subsequently the water in the membranes' tank was meticulously agitated during 45 min. Afterwards, filtration started at 24.5 LMH and after 2, 4, and 6 min both the membranes' tank (ie feed) and the permeate were sampled simultaneously. The microorganisms spiked in each integrity testing experiment were counted in 1 mL of the feed sample and in the case of the permeate in 1 L for bacteriophages and 10 mL for spores, according with the protocols described ahead.

2.3. Virus quantification

Enteroviruses (ENT) and noroviruses (NOR) in 1 L of raw river water were concentrated by organic flocculation, adding 3% of beef extract (BBL-Becton Dickinson, Sparks, MD) and adjusting the pH to 3.5 as described by Katzenelson et al. (1976).

Enteroviruses and noroviruses in pre-treated water (both direct UF permeate and dioxi-chlorinated, coagulated/flocculated and sand filtered samples) were concentrated by adsorption to and elution from positive charged cartridge filters MK-100 (AMF Corp., CUNO, Meriden, CT). For such purpose, 1000 L were filtered through MK cartridges. Viruses were eluted with 0.25 M glycine buffer solution at pH 10.5 for 25 min. A secondary concentration step was conducted by organic flocculation, adding 3% of beef extract (BBLBecton Dickinson, Sparks, MD) and adjusting the pH to 3.5 as described by Katzenelson et al. (1976). Prior to infection of the BGM cells, the concentrates were decontaminated and detoxified by filtration through 0.22 mm pore size low protein binding polyether sulphone membrane filters (Millipore, Bedford, MA).

Buffalo green monkey kidney cell line (ECAAC 90092601) was used for the enumeration infectious enteroviruses (ENT1). The method used for determination of PFUs was the double-layer plaque assay according to Mocé-Llivina et al. (2004). Detection limit was 0.2 PFU/L.

To quantify the genome copies (GC) of enteroviruses (ENT2), viruses in the concentrates described above were further concentrated and purified as described elsewhere (Hundesa et al., 2009;

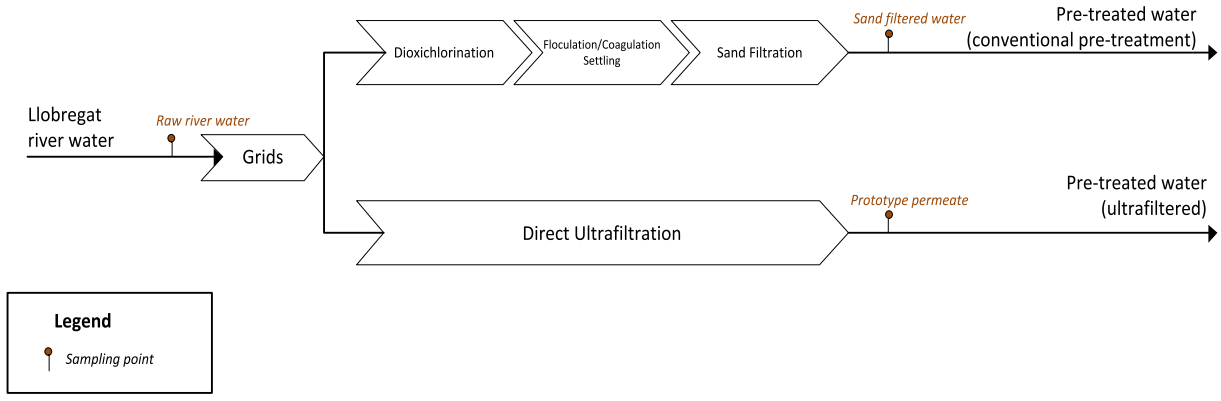

Fig. 1. Direct UF and conventional pre-treatment schemes compared within this study.

Puig et al., 1994). For the one-step quantitative real time RT-PCR method a set of primers and Taqman probe targeting a 155 bp of the 5′ untranscribed enteroviral genome region was used. The primers and probe sequences used to amplify and to detect viral genomes are described in Hwang et al. (2007) and Nijhuis et al. (2002), respectively. Briefly, the RT-PCR reaction was carried out in a total volume of 25 μL of nucleic acids extracted using a QIAamp viral RNA extraction kit (Qiagen, Madrid, Spain). The limit of detection of the technique was 35 Genome Copies (GC)/L of raw river water and sand filtered water and 0.75 GC/L in the ultrafiltered samples. Values in the detection limit were 350 GC/L in raw river and sand filtered samples and 50 GC/L in the ultrafiltered ones. In this case, values were calculated provided that 1000 L had been filtered.

To determine noroviruses GC, a standardized one-step real-time TaqMan RT-PCR using previously described primers and probes was employed for the detection of human noroviruses (NoVs) of genotype I (GI) (NoV GI) (da Silva et al., 2007; Svraka et al., 2007) and genotype II (GII) (NoV GII) (Kageyama et al., 2003; Loisy et al., 2005) as previously described by Pérez-Sautu et al. (2012). Virus/nucleic acid extraction and enzyme efficiencies were monitored as described elsewhere (Le Guyader et al., 2009), and used to estimate the actual genome copy numbers from the raw genome numbers measured by real-time RT-PCR in duplicate. The limit of detection was 16 GC/L of raw river water and sand filtered water and 0.8 GC/L in the ultrafiltered ones. In this case, values were calculated provided that 1000 L had been filtered.

2.4. Bacterial indicators quantification

E. coli were enumerated by membrane filtration using Chromocult® coliform agar (Merck, Germany) supplemented with antibiotics (*E. coli*/coliform selective supplement; Merck, Germany). Spores of sulfite-reducing clostridia (SSRC) were determined according to Bufton (1959). Spores of *Bacillus* sp in 10 mL aliquots of the samples were enumerated by pour plate technique using an adapted *Bacillus* specific medium (Yeast extract 20 g, acid-hydrolyzed casein 2 g, agar 15 g distilled H_2O_2 1000 mL) after a thermal shock at 80 °C during 10 min. Plates were incubated at 30 °C during 48–72 ± 3 h. One hundred mL were examined for the three bacteria studied in all types of samples; therefore the detection limit was 10 CFUs/L.

2.5. Bacteriophages quantification

Samples with expected low virus content were concentrated from 1 L of the water sample according to the method described by Mendez et al. (2004).

Plaque forming units (PFUs) of bacteriophages were counted after filtration of the sample through 0.22 mm pore size low protein binding polyether sulphone membrane filters (Millipore, Bedford, MA), and the phages were analyzed by the double agar layer technique. Somatic coliphages (SOMCPH) were enumerated on host strain *E. coli* WG5 in accordance with the standardized procedure (ISO, 2000) and F-specific RNA bacteriophages (FRNAPH), as well as bacteriophages MS-2 and GA on host strain *S. thyphimurium* WG49 according to ISO (1995). Bacteriophage PRD-1 was also enumerated by the double layer agar technique on strain *E. coli* MS1000 according to the procedures standardized for somatic coliphages (ISO, 2000). The volumes tested for bacteriophages in the different kinds of samples set up the detection limit at 1 PFU/L.

2.6. Physico-chemical parameters quantification

Both Llobregat River water and UF membrane permeate were characterized physico-chemically in a routine basis. The parameters monitored and the methods used were: turbidity by nefelometry (WTW GmbH VisoTurb 700IQ), total suspended solids (TSS) by ESS 340.2, absorbance at 254 nm (UV_{254}) by spectrophotometry (Hach-Lange DR 5000), silt density index (SDI) and modified fouling index (MFI) by ASTM D4189 (Simple SDI Meter 9C-281-0157) and dissolved organic carbon (DOC) by combustion-infrared method using a DOC analyzer (non-purgeable organic carbon, UNE-EN 1484), after filtration with a 1.2 μm glass fiber filter for the raw water samples (TOC-V CSH Shimadzu). When conducting membrane integrity tests, turbidity and UV_{254} were measured from feed and permeate samples (both unfiltered and filtered by 0.45 μm glass fiber filter in the case of the feed samples, and unfiltered for the permeate).

2.7. Data treatment

Logarithmic removal values (LRVs) were used to calculate the performance of the processes considered, calculated as shown in Eq. (1), being C_f the microorganism concentration in the feed stream and C_p the concentration in the permeate.

$$LRV = \log\left(\frac{Cf}{Cp}\right) \quad \text{Eq.1}$$

The software SPSS version 14.0 (SPSS Inc., Chicago, IL) was used to conduct the statistical analyses. Concentration values below the detection limit were taken as the detection limit for statistical analysis. This leads to calculate reductions equal or smaller than the actual reported values. For actual 0 values, the value 1 was used to calculate the logarithm. Differences were considered as significant at $P \leq 0.05$, as defined by the ANOVA or the Kursal–Wallis test.

Uncertainty analyses on the recovery of the assessed microorganisms were not conducted, although works addressing this issue can be found in literature (Wu et al., 2013).

3. Results and discusion

3.1. Llobregat River water characterization

Human viruses, bacteria and bacteriophages concentrations from the Llobregat River are reported in Table 1. The concentration of bacterial indicators in the studied sector of the Llobregat River are within the same order of magnitude than those of rivers of surrounding countries downstream of densely populated areas (Briancesco and Bonadonna, 2005; Cabral and Marques, 2006; Petit et al., 2001). Values missing are due to incidences in the testing procedure.

All the naturally occurring indicators considered were detected in all the samples with average values of *E. coli*, spores of sulfite reducing clostridia (SSRC) and somatic coliphages (SOMCPH) between 4.19 and 4.48 $\log_{10}$ units/L, and F-specific RNA bacteriophages (FRNAPH) averaging 1 $\log_{10}$ unit lesser. The values reported are similar to data previously published for the same Llobregat River section (Rubiano et al., 2012).

As shown in Fig. 2, only one value deviates more than 3-fold the standard variation of the data set. With the exception of this outlier value, all the other values are in between $\pm 3\sigma$ limits so that meaningful variations were not identified in the feed water quality regarding microbial indicators with the sampling campaigns conducted. Although it appears that there is a certain increase in the concentrations of the bacterial and viral indicators in the second half of the sampling period, the difference is not significant (ANOVA, $P > 0.05$) for any of the indicators considered.

In terms of human viruses, infectious enteroviruses (ENT1) were

Table 1
Concentrations of bacterial and viral indicators, and human viruses expressed in $\log_{10}$ units per liter of raw river water sample. Units are CFUs for *E. coli* and SSRC; PFUs for SOMCPH, and FRNAPH and ENT1; and GC for ENT2, NoV GI and NoV GII.

	Positive samples	*Mean (log_{10}unit/L)*	Standard deviation *(log_{10}unit/L)*	Minimum *(log_{10}unit/L)*	Maximum *(log_{10}unit/L)*
Indicator					
E. coli (CFU)	46/46	4.28	0.49	3.00	5.71
SSRC (CFU)	47/47	4.19	0.35	3.48	5.10
SOMCPH (PFU)	47/47	4.48	0.37	3.81	5.54
FRNAPH (PFU)	46/46	3.15	0.66	2.30	4.56
Virus					
ENT1 (PFU)	1/7	–	–	<0.20	0.62
ENT2 (GC)	6/6	4.69	0.86	3.30	5.77
NoV GI (GC)	6/6	4.20	1.63	2.90	6.03
NoV GII (GC)	7/7	4.65	1.09	3.13	5.82

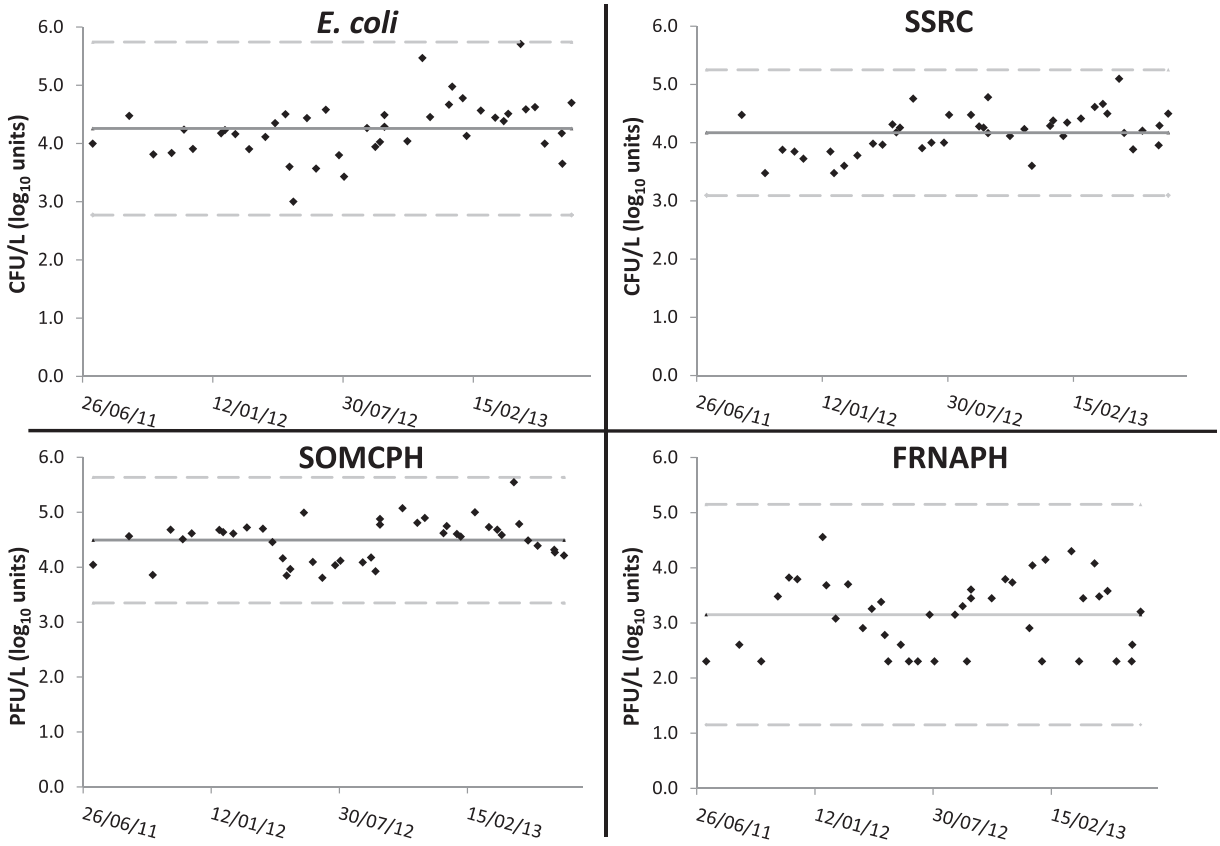

Fig. 2. Concentration evolution along time of *E. coli*, SSRC, somatic coliphages SOMCPH and FRNAPH in the river water samples. Center line (solid) corresponds to the data mean, and the upper and lower lines (dotted) to the mean ± 3σ standard error, respectively.

only found in 1 of 7 samples tested, which is similar to what has been described in Rubiano et al. (2012). In contrast, genomes copies (ENT2, NoV GI and NoV GII) were detected by q-RT-PCR in all samples tested. Values in CG $\log_{10}$ units/L ranged from 3.30 to 5.77 for enteroviruses and from 2.90 to 6.03 and from 3.13 to 5.82 for noroviruses genotype I and II respectively. Values for noroviruses are comparable to those described previously in the same river section (Pérez-Sautu et al., 2012). No previous values of GC of enteroviruses are available for the Llobregat River but the concentrations determined are comparable to the ones found in river water samples collected in France with concentrations of *E. coli* similar to the ones reported herein (Schvoerer et al., 2001).

The Llobregat River physico-chemical parameters for the considered period are summarized in Table 2. Despite the variability of the raw river water, the quality of the permeate produced within the direct UF scheme was stable, with turbidities consistently below 0.09 NTU and TSS under 1.0 mg/L. Taking into account the high inlet turbidity changeability (8–>1000 NTU), the low

Table 2
Average ± confidence interval (significance of 0.05) of the Llobregat River and UF permeate physico-chemical parameters assessed during May 2011–July 2013. HL: high level (i.e. more than 5%/min); a.u.: absorbance units.

Parameter	Unit	Raw river water	UF permeate water
Turbidity	NTU	169.3 ± 61.9	0.08 ± 0.005
TSS	mg/L	153.03 ± 58.72	0.84 ± 0.14
UV_{254}	a.u.	0.1105 ± 0.0075	0.0861 ± 0.0025
DOC	mg/L	6.19 ± 1.30	4.03 ± 0.26
SUVA	L/mg	2.34 ± 0.21	2.21 ± 0.08
SDI	%/min	HL	1.76 ± 0.44
MFI	s/L^2	2534.5 ± 522.1	0.37 ± 0.44

variability of the UF permeate demonstrates the reliability of the process in terms of physico-chemical parameters, being independent of feed water quality. The average removal rates for turbidity, TSS, UV_{254} and DOC were 99.4 ± 0.1%, 97.1 ± 1.1%; 15.31 ± 2.06% and 16.94 ± 4.81% (confidence level of 0.05), respectively for the direct UF scheme. Further details of the prototype performance from a hydraulic and physico-chemical point of view can be found in Galvañ et al. (2014).

3.2. Microbial elimination by direct ultrafiltration and conventional pre-treatment scheme

3.2.1. Indicator pathogens presence in the treated water

Presence and numbers of indicators and human viruses in the conventional pre-treated (dioxi-chlorination, coagulation/flocculation, settling and sand filtration) water are listed in Table 3 and its evolution along time is plotted in Fig. 3. Both bacterial and viral indicators were found in a number of samples ranging from 5 out of 34 for FRNAPH (the less abundant) and 28 out of 35 for SSRC (the most abundant). Maximum values ranged from 0.85 $\log_{10}$ PFU/L for FRNAPH to 2.9 $\log_{10}$ CFU/L for SSRC.

Occurrence and concentrations of indicators and human viruses in ultrafiltered water are summarized in Table 4, and its evolution along time in Fig. 4. Regarding bacterial indicators, *E. coli* was never found, whereas SSRC were found in 2 out of 40 samples, with a maximum value of 1.48 $\log_{10}$ CFU/L. Taking into account the larger size of bacterial indicators compared to the membrane pores, their removal would be expectable by sieving effect. Therefore, these results point out a good performance of the prototype in terms of removal of bacterial indicators, always greater than 500 nm, and hence, greater than the pore size of the UF membrane. Analogously to the physico-chemical parameters (Galvañ et al., 2014), a relationship between peak indicator concentrations in the ultrafiltered water and in the raw river water was not found. In contrast, bacteriophages, mostly somatic coliphages, were present in an important fraction (60%) of the direct UF samples, with a maximum value of 1.80 $\log_{10}$ PFU/L. F-specific RNA phages were present in 20% of the samples presenting a maximum value of 0.80 $\log_{10}$ PFU/L. Consequently, it can be concluded that a significant fraction of phages cross the membrane. F-RNA are smaller (20–25 nm) than the pore size, whereas most of the coliphages are greater than 40 nm. However, a fraction of them, estimated to be between 1 and around 10% in sewage and river water samples (Muniesa et al., 1999) are *Microviridae* with a size of 20–25 nm and hence, similarly to F-RNA bacteriophages, are smaller than the pore size. This fraction is very likely to account for the SOMCPH that pass through the membrane.

The percentage of positive samples for SSRC was significantly greater (Krustal–Wallis tests, $P < 0.05$) in the sand filtered samples (Table 3) than in the ultrafiltered samples (Table 4). In the case of *E. coli* it was also greater but not significantly, probably the fact that all *E. coli* samples were negative and that the detection limit was used for the calculations influence this lack of significance. The percentage of positive samples for bacteriophages was slightly, but not significantly (Krustal–Wallis tests, $P < 0.05$), higher in the ultrafiltered samples; again this lack of significance may be due to the low number of positive samples and to the fact that the detection limit was used for the calculations. In the conventional pre-treatment, the removal of indicators is due to two processes: first, the removal caused by coagulation/flocculation - settling - sand filtration and secondly, the inactivation by chlorine dioxide. Then, it is not surprising that the SSRC that are known to be quite resistant to chemical disinfection (Venczel et al., 1997) were found in the sand filtered samples much more frequently than the other indicators. These results suggest that inactivation by chlorine (rather than the retention by sand filtration) plays the most important role in the removal of indicators.

Some conventionally pre-treated water samples contained indicators, both bacterial and viral, whereas some ultrafiltered water samples contained bacteriophages but never bacterial indicators (Figs. 3 and 4, respectively). The treated samples containing indicators did not correspond to sampling days with greater indicators concentrations in river water. Therefore, the presence of indicators in treated water samples should be attributed to the treatments themselves and not to increased indicators concentrations in the river. Additionally, with the available data, no trend is observed in terms of indicator concentrations evolution along time in the treated samples from both processes.

No infectious enteroviruses (ENTPFU) were found either in the sand filtered water or in the ultrafiltered samples (Tables 3 and 4). In contrast, CG of enteroviruses and noroviruses were present in half of the sand filtered samples. This is not unexpected since the q-RT-PCR has been described to poorly perceive the effects of disinfection processes: very slight reductions were quantified by GC counts detected by PCR in comparison to those quantified by the infectious viruses (Sobsey et al., 1998; Simonet and Gantzer, 2006). Finding GC copies of a human virus in a given water sample does not mean that they pose a risk for human health (Gassilloud et al., 2003) because they may not be infectious. As a result, the infectious enterovirus and/or bacteriophage indicators measurement would be more appropriate than detecting genomes to assess the viral quality of the water for a DWTP when chemical or UV disinfection is included in the treatment process. Additionally, it is important to point out that these values correspond to pre-treated water, still pending further treatment steps (e.g. RO) which fully guarantee the water quality distributed by SJD DWTP according to the existing legislation.

Table 3
Concentrations of bacterial and viral indicators, and human viruses expressed in $\log_{10}$ units per liter of treated water in the conventional pre-treatment scheme (dioxi-chlorination – coagulation/flocculation – settling – sand filtration. Units are CFUs for *E. coli* and SSRC; PFUs for SOMCPH, and FRNAPH and ENT1; and GC for ENT2, NoV GI and NoV GII.

	Positive samples	*Mean ($\log_{10}$unit/L)*	Standard deviation *($\log_{10}$unit/L)*	Minimum *($\log_{10}$unit/L)*	Maximum *($\log_{10}$unit/L)*
Indicator					
E. coli (CFU)	10/34	1.07	0.20	1.00	1.95
SSRC (CFU)	28/35	1.56	0.51	1.00	2.90
SOMCPH (PFU)	10/35	0.25	0.47	0.00	1.59
FRNAPH (PFU)	5/34	0.06	0.18	0.00	0.85
Virus					
ENT1 (PFU)	0/6	–	–	<0.20	<0.20
ENT2 (GC)	4/6	2.27	2.27	<1.54	1.96
NoV GI (GC)	3/6	2.54	2.54	<1.20	5.52
NoV GII (GC)	3/6	2.22	2.22	<1.20	4.95

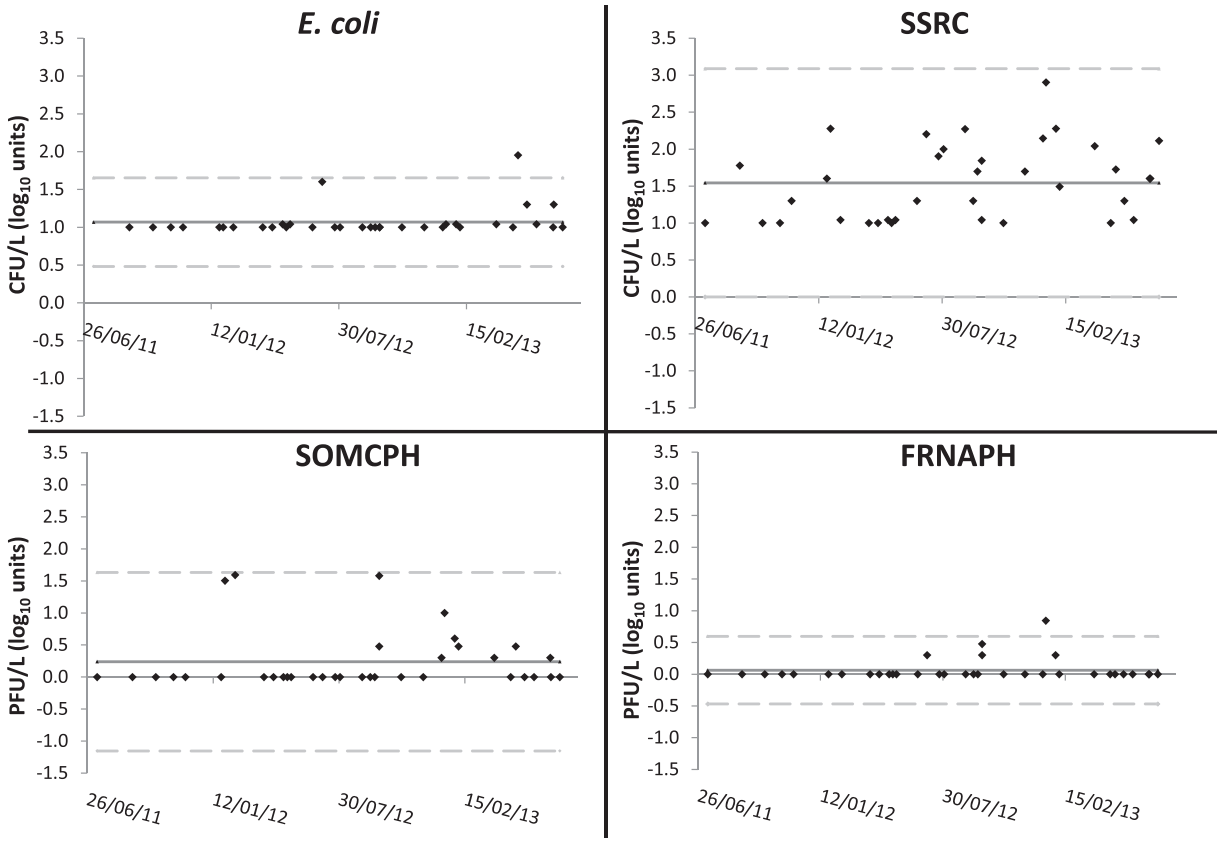

Fig. 3. Concentration evolution along time of *E. coli*, SSRC, somatic coliphages SOMCPH and FRNAPH in pre-treated water samples with the dioxichlorination – coagulation/flocculation-settling-sand filtration treatment scheme. Center line (solid) corresponds to the data mean, and the upper and lower lines (dotted) to the mean ± 3σ standard error, respectively.

3.2.2. Indicator pathogens' reductions achieved by each treatment scheme

Fig. 5 shows the average (±standard error) of the reductions achieved by the direct UF prototype and the conventional pre-treatment. As indicated in the methods section, the LRVs plotted here are smaller than the actual ones, since the values used to calculate them are the detection values when detection is negative. Nevertheless, some conclusion can be drawn from these results. Average reductions of bacterial indicators achieved by the direct UF scheme were greater than 4 $\log_{10}$ units and those of viruses range from greater than 3 $\log_{10}$ units (FRNAPH) to greater than 3.9 (genome copies of Noroviruses II).

Regarding bacterial indicators, reductions achieved by direct UF were significantly (ANOVA, $P < 0.05$) greater than those attained by the conventional pre-treatment. In contrast, reductions of viral indicators were larger in the conventional pre-treatment. Though this is observed with both groups of bacteriophages only the reductions of SOMCPH achieved by the conventional pre-treatment were significantly (ANOVA, $P < 0.05$) superior than those achieved by direct UF.

In terms of human viruses genome copies (GC), the reductions achieved by direct UF were significantly (ANOVA, $P < 0.05$) greater than those achieved by the conventional pre-treatment, and similar (ANOVA, $P > 0.05$) to those achieved for bacteriophages by direct UF. These low reductions, less than 2 $\log_{10}$ units of GC of enteroviruses and noroviruses, can be due to the fact that GC counts detected by PCR use to experience very slight reductions when submitted to chemical disinfection in comparison to the decreases experienced by the infectious viruses (Sobsey et al., 1998; Simonet and Gantzer, 2006).

Table 4
Concentrations of bacterial and viral indicators, and human viruses expressed in $\log_{10}$ units per liter of ultrafiltered water. Units are CFUs for *E. coli* and SSRC; PFUs for SOMCPH, and FRNAPH and ENT1; and GC for ENT2, NoV GI and NoV GII.

	Positive samples	*Mean ($\log_{10}$unit/L)*	Standard deviation *($\log_{10}$unit/L)*	Minimum *($\log_{10}$unit/L)*	Maximum *($\log_{10}$unit/L)*
Indicator					
E. coli (CFU)	0/39	<1.00	0.00	<1.00	1.00
SSRC (CFU)	2/40	<1.01	0.07	<1.00	1.48
SOMCPH (PFU)	24/40	<0.55	0.52	<0.00	1.80
FRNAPH (PFU)	8/40	<0.08	0.18	<0.00	0.85
Virus					
ENT1 (PFU)	0/7	–	–	<0.20	<0.20
ENT2 (GC)	2/6	–	–	<0.12	1.96
NoV GI (GC)	1/7	–	–	<0.09	0.22
NoV GII (GC)	1/7	–	–	<0.09	0.19

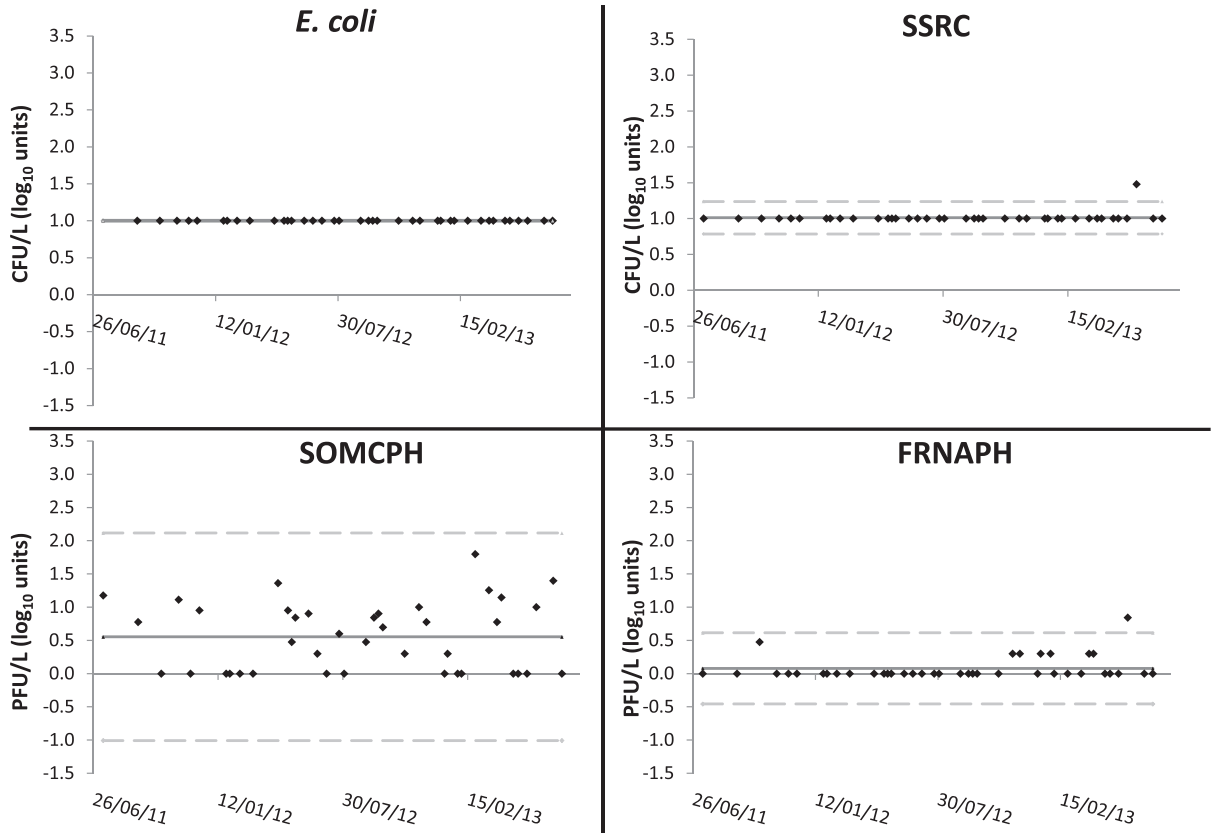

Fig. 4. Concentration evolution along time of *E. coli*, SSRC, SOMCPH and FRNAPH in the ultrafiltered water (direct UF treatment scheme). Center line (solid) corresponds to the data mean, and the upper and lower lines (dotted) to the mean $\pm 3\sigma$ standard error, respectively.

A relationship between the LRVs of any of the microorganisms considered within this project and the membranes' transmembrane pressure (TMP), which was monitored when doing the microbiological sampling, was not found (data not shown). Nonetheless, the low number of positive microbiological samples and the fact that the detection limit was used to calculate the microbiological LRVs could mask the presence of such relationship, if existing.

3.3. Direct ultrafiltration membrane integrity tests

Seven experiments distributed evenly during two years were undertaken to assess the direct UF membrane integrity. The concentration of the seeding organisms were kept between 10^3–10^4 PFU/mL or CFU/mL for GA, MS-2 and *Bacillus* spores and between 10^2 and 1.7×10^3 PFU/mL for PDR-1in the feed tank. Table 5 summarizes the results obtained in terms of LRVs. No

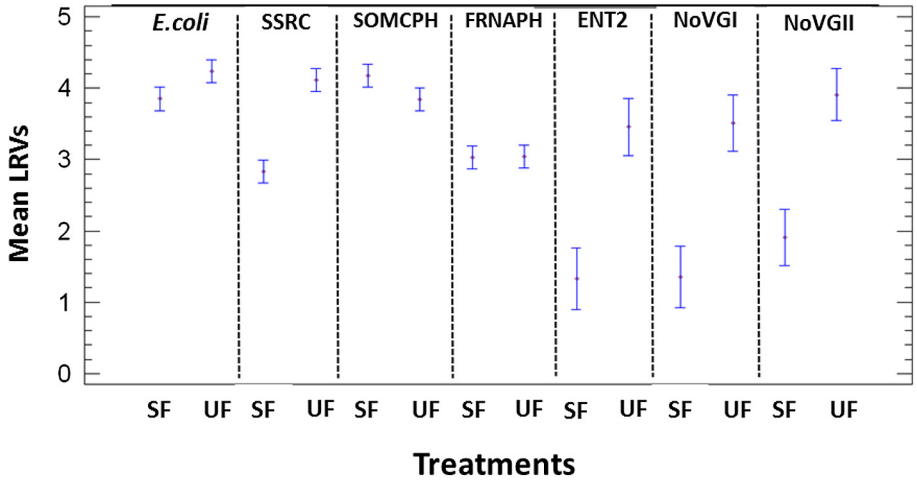

Fig. 5. Mean LRVs of *E. coli*, spores of sulfite reducing clostridia (SSRC), somatic coliphages (SOMCPH), F-specific RNA coliphages (FRNAPH) ENT2, NoV GI and NoV GII, for direct UF (UF) and conventional pre-treatment, (SF); schemes obtained during the two year period considered.

Table 5
LRVs of the numbers of PFU/mL of GA, MS-2 and PDR-1, and CFU/mL of *Bacillus* spores achieved during the membrane integrity monitoring experiments performed in the direct UF treatment scheme during a two year period.

Indicator	Positive detections in the permeate	LRV mean	LRV st dev.	LRV minimum	LRV maximum
GA	7/7	3.02	0.23	2.70	3.19
MS-2	7/7	2.81	0.31	2.34	2.81
PDR-1	6/7	>5.00	1.24	3.69	>6.22
Bacillus spores	2/7	>5.10	1.05	3.86	>5.89

evident temporal trend was observed.

As shown in Table 5, MS-2 and GA reductions are similar (ANOVA, $P > 0.05$), flanking 3 $\log_{10}$ units. Since both bacteriophages are fairly smaller than the pore size, they are not expected to be retained by size exclusion, but by adsorption and static interactions. MS-2 and GA have been considered as extreme cases in terms of membranes' absorbability, since they have different isoelectric point (MS-2: 3.1–3.9 and GA: 2.1–2.3) and hydrophobicity (GA > MS-2) (Langlet et al., 2008). Consequently, their use is considered to surrogate the behavior of the great majority of human viruses. In conclusion, this set of experiments shows that concentrations of all the human viruses will be reduced by 3 $\log_{10}$ units by direct UF with a membrane like the one used in these experiments. These results are comparable to the LRV reported for naturally occurring bacteriophages analyzed in the routing sampling campaigns reported herein and values published in literature (Zhu et al., 2005; Zodrow et al., 2009).

PDR-1 (60 nm in size) and *Bacillus* spores (length > 1000 nm; diameter > 500 nm) removals were above 5 $\log_{10}$ units (Table 5), significantly different to the abovementioned bacteriophage removals (ANOVA, $P > 0.05$). These micro-organisms are removed by size exclusion. PDR-1 can go through the membrane either by the existence of pores greater than the nominal size or by failures in membrane integrity, whereas*Bacillus* spores only can go through by failures in membrane integrity. The presence of *Bacillus* spores in the permeate could be due to some environmental concentration because it has been reported that they are present in DWTP environments (Galofré et al., 2004). However, because the routine monitoring quantified some spores of *Clostridium* in the permeate and because LRVs of PDR-1 and *Bacillus* spores are similar, its presence in the permeate could be due to a small leakage in the UF system or some minor water by-pass in the prototype system.

The similarity between the LRVs of bacteriophage PDR-1 and *Bacillus* spores indicates that the pore size distribution of the membrane is within the average indicated by the supplier and that the fraction of pores greater than 60 nm is rather low or inexistent.

The LRVs calculated for the three bacteriophages and *Bacillus* spores do not diminish along the whole 2 year period. This indicates, on one hand that membrane integrity has not changed, and on the other, that the properties of the membranes that affect the bacteriophages removal have not changed. As commented previously, besides size exclusion, adsorption and electrostatic interactions can also lead to virus/bacteriophages removal. Among the various factors already discussed that affect virus adsorption onto pores larger than their size, those related to feed water quality have been kept constant during the assays performed thanks to the protocol defined and thus, only the ones related to membrane properties (e.g. irreversible fouling, structure, integrity, composition) could have affected the results. The relationship between the bacteriophages and *Bacillus* spores removal during the integrity tests and the membrane TMP or the permeability divided by the pressure (temperature corrected) has been assessed, but no relationship has been observed (data not shown). As a result, it can be concluded that the membrane has not been modified due to operation in such a way that its behavior in terms of bacteriophages rejection has been altered, despite some irreversible fouling may have been accumulated. This also indicates that the operation of the UF membrane during the 2 years period did not produce damages driving to a loss of integrity, despite the harsh conditions it faced in terms of water quality and continuous operation.

Finally, the comparison between the bacteriophages and the *Bacillus* spores LRVs with water physico-chemical parameters (data not shown of UV_{254} and turbidity measured during the integrity tests both in the feed and permeate) has shown that these measurements cannot be used as surrogates of the UF membrane integrity tests based on microorganisms considered in this work. No relationship between their removal and the LRVs of GA, MS-2, PDR-1 or *Bacillus* spores has been found, reinforcing the usefulness of these microbes, especially bacteriophage PRD-1, for membrane integrity tests targeting UF membranes whose pore sizes are greater than 20 nm.

4. Conclusions

Based on the results obtained in this work, it can be concluded that the direct UF scheme tested ensures the average removal of more than 5 $\log_{10}$ units of bacteria and viruses greater than 60 nm. In the case of microbes of this size, the performance of the assessed UF membrane significantly outperforms that of the dioxichlorination, coagulation/flocculation, settling and sand filtration pre-treatment, presenting greater removal values and lower variability. In contrast, the direct UF scheme only guarantees a 3 $\log_{10}$ units removal of viral indicators and viruses whose sizes are lesser than 40 nm. For microbes of this size the performance of the dioxichlorination, coagulation/flocculation, settling and sand filtration (current pre-treatment) is better than that of direct UF membrane assessed (PVDF 40 nm in pore size), although not always significantly. According to the results obtained, the removal of the microbiological parameters assessed does not depend on their feed water concentration, analogously to the physico-chemical parameters assessed. Additionally, a relationship between the TMP and/or the permeability of the membrane and the human virus, bacterial and viral indicators rejection has not been found, so that fouling effects on microbial performance have not been observed within this work, which has covered a two year period.

The implementation of direct UF has proved to be a feasible alternative to conventional pre-treatment, being particularly advantageous regarding bacterial indicators and human viruses genome copies removal. As envisaged, the treatment of the Llobregat River water with direct UF would still require some further treatment to fully eliminate the viruses. However, in both cases, the current conventional pre-treatment and the direct UF scheme have/would have subsequent stages to ensure the final quality, fulfilling the drinking water quality standards. As a result, the substitution of DWTPs conventional pre-treatment will depend on several factors besides microbiological performance, such as space requirements, reagents dosage, waste generation and energy requirements, being the three first advantageous in the direct UF scheme, as described in complementary previous works (Galvañ et al., 2014).

The results presented here confirm other results regarding the usefulness of determining infectious virus to follow the effectivity of chemical disinfection processes compared to PCR genome copies, since not all genome copies are infectious and hence, represent a risk for human health.

Both naturally occurring viral indicators studied (somatic coliphages and F-specific RNA bacteriophages) are removed by direct UF similarly to human viruses when measured as GC, and similarly to seeded small bacteriophages (GA and MS-2). They can be suggested to be used to follow up the performance of UF membranes in terms of virus removal. Additionally, taking into consideration the large numbers found in the raw river water and in the permeate, as well as the straightforwardness of the standardized enumeration method (ISO, 2000), SOMCPH could be considered as a good tool for surveying the performance of UF membranes with indicators present in the raw river water. In this case, reductions between 3.0 and 4.0 $\log_{10}$ units of the numbers of somatic coliphages would be expectable.

A main concern related to membrane-based processes is its ageing and the effects that this can provoke in terms of membrane integrity and thus, the quality of the permeate produced. The monitoring of the microbiological data obtained indicates that a perceptible loss of integrity of the tested membrane has not occurred. This means that dealing with raw river water directly of highly variable quality (turbidity ranging from 8 to >1000 NTU) continuously during 2 years has not compromised the membrane (by physical and/or chemical means).

Bacteriophage PDR-1 and *Bacillus* spores appear as suitable microbes to monitor membrane integrity. Bacteriophage PDR-1 is easier to grow to high densities than *Bacillus* and therefore it would be a better option than MS-2 and GA bacteriophages in some cases. However, obtaining sufficient amounts of phages to seed a full water treatment process is still challenging. Turbidity and UV_{254} measurements conducted during membrane integrity tests have not presented a relationship with the microbes assessed during these tailored tests, so that they have not been able to provide equivalent information within this work.

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