Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.795339

51 (2013) 4947–4957
July

Taylor & Francis
Taylor & Francis Group

Challenge tests with virus surrogates: an accurate membrane integrity evaluation system?

O. Ferrer[a], R. Casas[b], C. Galvañ[a], F. Lucena[b], A. Vega[c], O. Gibert[a,d], J. Jofre[b], X. Bernat[a,*]

[a]*CETaqua, Water Technology Center, Carretera d'Esplugues 75, Cornellà del Llobregat 08940, Spain*
Email: xbernat@cetaqua.com
[b]*Departament de Microbiologia, UB, Universitat de Barcelona, Av. Diagonal, 643, Barcelona 08028, Spain*
[c]*SGAB, Sociedad General de Aguas de Barcelona, Av. Diagonal 211, Barcelona 08018, Spain*
[d]*Departament d'Enginyeria Química, ETSEIB, Universitat Politècnica de Catalunya, Av. Diagonal 647, Barcelona 08028, Spain*

Received 6 September 2012; Accepted 18 March 2013

ABSTRACT

The use of membrane filtration in drinking water treatment has significantly increased in the last decades due to its advantages, including its capacity to produce water of high quality with a high level of pathogens rejection. However, if membrane integrity is compromised, this feature cannot be guaranteed, increasing the associated microbial risk of the treated water. This study has focused on the development and application of a protocol based on virus surrogates challenge tests applicable to the three existing ultrafiltration (UF) configurations. The operational conditions have been defined, and the tests have been conducted successfully. The selected micro-organisms, PDR-1, MS-2, GA and *Bacillus* spores, present different characteristics providing complementary information of membrane integrity and its status. In particular, PDR-1 and *Bacillus* spores, due to their larger size, are mainly removed by size exclusion and low removal rates may indicate membrane impairment. MS-2 and GA, 25 nm in size approximately, may not be rejected by size exclusion but by adsorption and electrostatic interactions, so that their removal values may not necessarily be indicative of membrane integrity failures. Since they may be influenced by further factors, such as membrane characteristics, feed water quality, non-chemically removable fouling, etc., the results obtained can be used to better understand membranes performance.

Keywords: Membrane integrity; Viruses surrogates; Bacteriophages; *Bacillus* spores; *Cryptosporidium*; Ultrafiltration; Pressure decay test

*Corresponding author.

Presented at the Conference on Membranes in Drinking and Industrial Water Production.
Leeuwarden, The Netherlands, 10–12 September 2012.
Organized by the European Desalination Society and Wetsus Centre for Sustainable Water Technology

1. Introduction

During the last decades, membrane technology has significantly evolved becoming a technological solution increasingly applied in drinking water treatment plants (DWTPs) due to its advantages. Among them, it is remarkable that its capacity to produce high-quality treated water independently of incoming water quality fluctuations. High levels of pathogens rejection can be achieved, as demonstrated by several researchers who proved a complete removal of coliform bacteria, *Giardia* spp. and *Cryptosporidium* spp. by low-pressure membranes, which include microfiltration (MF) and ultrafiltration (UF) [1]. Due to the lower cut-off of UF membranes compared with MF ones, the former present some disinfection capacity, being able to remove a percentage of viruses as can be seen in Fig. 1. The high-quality water obtained by membrane processes, linked to their reliability, modularity (and thus, easiness to scale-up), reduced space requirements, operation easiness, possibility of being automatised and relatively low cost has led to their recognition as promising processes for water treatment. Consequently, the globally installed volume of low-pressure membrane systems has increased almost exponentially [2], being 60% of its applications for drinking water treatment [3].

Although MF or UF membranes represent a theoretical absolute physical barrier to particles (pathogens, indicators, suspended solids, etc.) that are larger than the membrane pore size, they can suffer damages that lead to a significant decline in permeate quality. Therefore, if membrane integrity is compromised, pathogen will pass through it and thus contaminate the product water. Failure of membrane fibres may be due to several reasons: (i) chemical corrosion (e.g. oxidation); (ii) defective installation and maintenance; (iii) operational conditions causing membrane stress and strain (e.g. backwash (BW), vigorous bubbling); (iv) impairment by sharp objects not removed in the pre-treatment [1]. Zondervan et al. [4] identified several aspects that may act as significant ageing agents and hence contribute to membrane failure: membranes fouling degree, the number of applied back pulses and the combination of these two factors.

Membrane integrity is a critical aspect for any membrane filtration plant in order to minimise its associated microbial risk. The minimisation of microbial risk involves demonstrating that the membrane system can adequately remove pathogens and the periodic and/or continued verification that the membrane is intact. The United States Environmental Protection Agency (US EPA) established through different pieces of legislation surface water treatment rule (SWTR) [6]; interim-enhanced surface water treatment rule (IESWTR) [7]; long-term 1-enhanced surface water treatment rule (LT1ESWTR) [8]; long-term 2-enhanced surface water treatment rule (LT2ESWTR) [9]) target removal values for the whole water treatment process as well as for the membrane-based filtration process for certain pathogens. Additionally, this organisation defined the testing procedures to be implemented to validate the log removals of membrane-filtration

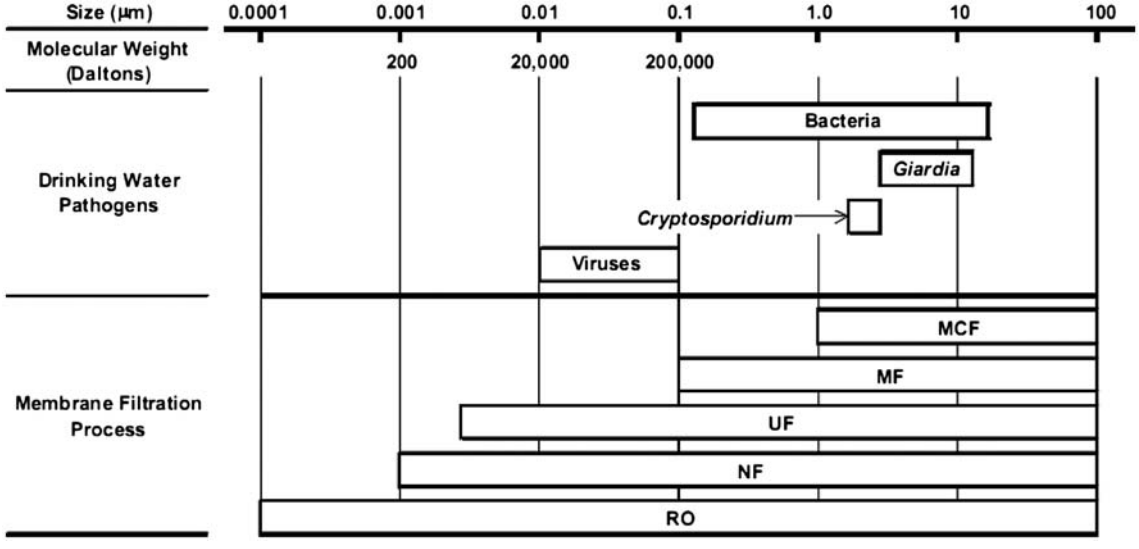

Fig. 1. Approximate retention spectrum of different water borne pathogens and membrane filtration processes capacity to remove them, based only on size exclusion. NF: nanofiltration; RO: reverse osmosis; MCF: membrane cartridge filtration [5].

processes (challenge tests, conducted in the framework of the environmental technology verification (ETV) programme) and the surveillance tests (direct integrity testing and continuous indirect monitoring) to be conducted to ensure the log removals previously obtained in the challenge tests. Integrity testing represents a practical way of verifying the barrier effectiveness by detecting leaks or membrane breaches and can be measured by direct and indirect methods [1], such as the quantification of certain pathogens (or their surrogates) removal rates. However, membrane integrity tests have to be adapted to each specific membrane and, in the case of surrogates challenge tests, testing conditions are not sufficiently defined in the United States EPA membrane filtration guidance manual [5].

Due to these limitations, this study has focused on the development and application of a membrane integrity testing protocol applicable to the three basic configurations of UF technology (pressurised inside-out, pressurised outside-in and submerged outside-in). For such purpose, different surrogates have been selected and appropriate testing conditions have been defined, aiming at determining the most convenient model organism and the testing operational settings. The results obtained have been compared with conventional pressure decay tests (PDTs) to assess the reliability and accuracy of the approach adopted. The limitation of the PDT tests has been reported in the literature, concluding that plants relying solely on this method may work beyond the point where the integrity is compromised because they cannot guarantee viruse removal [1]. The pressure used in the PDTs determines the minimum defect size to be identified. For a defect size of 3 μm, as stipulated in the LT2ESWTR to be achieved by membrane processes, the pressure needed, adopting a conservative approach, would be 1 bar, for 1 μm defect 3 bar, for 0.1 μm defect 30 bar and for 0.02 μm defect (virus size) 150 bar. Therefore, to identify defects below 1 μm, the pressures required are excessive and unviable to be implemented in UF systems.

Membrane integrity monitoring based on permeate characteristics such as turbidity or particle counting enables a continuous follow-up of the membrane systems. Nevertheless, the identification of permeate quality decrease typically requires a significant impairment of the membrane, so that more precise tools are needed to complement these routine testing.

2. Methodology

2.1. Surrogates selection and quantification

Three different bacteriophages have been selected to undertake this work: MS-2, GA and PDR-1, as well as *Bacillus* spores as a control due to its larger size. The latter can be considered as a surrogate of *Cryposporidium* [10–12]. Bacteriophages have been selected because they are similar to enteric viruses (phages share many properties with human viruses in terms of composition, structure, morphology and capsid size), and they are innocuous to humans and high titres can be obtained in the laboratory. MS-2 and GA have the same size (25 nm approximately) but differ in isoelectric point and hydrophobicity, presenting a different tendency to adsorb onto solids and to aggregate [13]. In particular, they are considered the extreme cases in terms of membranes adsorbability, so that the behaviour of the great majority of human viruses in this respect will be in between MS-2 and GA. PDR-1 has a size of 60–70 nm, and *Bacillus* spores about of 900–1,600 nm. The surrogates retention mechanisms that will take place excluding size (for those surrogates whose size is greater than the membranes pore sizes), adsorption and electrostatic forces.

Quantifications of *Bacillus spores* in 10 mL aliquots of the samples have been enumerated by pour plate technique using an adapted *Bacillus*-specific medium after a thermal shock at 80 ℃ during 10 min. Plates have been incubated at 30 ℃ during 48 to 72 ± 3 h. The limit of detection of the technique is 0.1 colony-forming units (CFUs) per mL. Regarding bacteriophages quantification pre-filtered samples have been quantified without bacteriophages concentration. In the case of UF permeate samples, bacteriophages have been concentrated. Bacteriophages have been enumerated by the double-agar-layer method described by [14]. GA and MS-2, which are F-specific RNA bacteriophages, have been enumerated according to ISO standard 10705-1 [15]; and PDR-1, which is a somatic coliphage, according to ISO 10705-2 [16], but using host strain E. Coli MS1000 instead of *E. Coli* WG5 used for somatic coliphages. The limit of detection of the technique is 1 plaque-forming unit (PFU) per mL in pre-filtered samples and 0.001 PFUs per mL in the UF permeates.

The logarithmic removal values (LRV) obtained, calculated by Eq. (1), provide information about the integrity of the membrane fibres. C_f and C_p represent the concentration of the surrogate under consideration in the feed and permeate stream, respectively. C_f is also referred as the spiked concentration and denotes the surrogates concentration that the membrane faces in each challenge test.

$$\mathrm{LRV} = \log\left(\frac{C_f}{C_p}\right) \tag{1}$$

The LRV awarded to each membrane in each challenge test has been calculated as the arithmetic mean

of the three LRV obtained at the three different sampling times of each experiment.

LRV for *Bacillus* spores have been calculated theoretically, apart from experimentally measured, based on LT2ESWTR Eq. (2), which relates the pressure drop recorded during the PDT with the removal rates. Q_p stands for the permeate flow, Q_{breach} the flow passing through an impaired fibre and VCF the volumetric concentration factor.

$$\text{LRVt} = \left(\frac{Q_p}{\text{VCF}Q_{\text{breach}}}\right) \quad (2)$$

Full-scale membrane modules with different pore sizes (ranging from 20 to 40 nm) and characteristics have been used to assess the suitability of the method developed. The tests have been applied every three months approximately to the same membrane modules, which have been in continuous operation for direct surface water pre-treatment demonstration, to assess their integrity and changes suffered over time. When data are not shown in the graphs, it is due to an operational or analytical problem so that results have not been obtained.

2.2. Challenge test protocol definition

The test consists in undertaking a chemically enhanced backwash (CEB) to the membrane, adjusting the feed water (dechlorinated tap water) conductivity, inoculating a known stock concentration of bacteriophages/spores and evaluate the concentration over time both in the feed and in the permeate streams during filtration. It is important to conduct the test without coagulant dosage (some coagulants entrap and adsorb in/on flocks and present virucidal activity, such as the aluminum-based ones [17–19] nor pH correction because of its effect on adsorption [17,18].

The CEB is performed following the recommendations of the membrane manufacturer in terms of chemicals used, concentration, stages applied and duration. The aim of the preliminary chemical cleaning is to remove the membrane deposits and hence leave the membrane defects, if existing, uncovered. Feed water tank is cleaned and tap water is introduced and dechlorinated by adding sodium thiosulphate (0.1 mol/L; J.T. Baker). Sodium chloride (99.0% purity; J.T. Baker) is then added to the feed water tank to adjust its conductivity to 2,000 μS/cm. As a result, the ionic strength of the testing solution is kept constant between experiments and it can be achieved in each challenge test trial.

After 10–30 min of feed water tank stirring (this value depends on the characteristics of the testing facility used), the system is forced to filter for 2 min (this value is based on the characteristics of the testing facility used as well) in order to flush the piping and determine the initial membrane resistance, which enables the establishment of the fouling level of the membrane. Bacteriophages and/or spores are then spiked in the feed tank. Due to the characteristics of the detection methods of the surrogates used, MS-2 is spiked together with PDR-1 and GA with *Bacillus* spores. The protocol has thus to be applied twice. The seeding concentration of the surrogates has been selected in such a way to prevent surrogates aggregation, which would overestimate the system removal capacity, as recommended by the LT2ESWTR [9].

Feed water tank agitation for 20–45 min (this value depends on the characteristics of the testing facility used) is applied to ensure homogeneous solution of the surrogates in the feed tank. Subsequently, the system starts filtration at a given constant flow (30–40 L/(m^2 h) depending on the membrane), without applying any BW nor CEB cycle. The filtration flux has been selected below the maximum nominal value of each tested membrane, because these tests are planned to be carried out during the membrane lifetime. Therefore, in case of suffering severe non-chemically removable fouling, it may be difficult to achieve or sustain a high flow during the challenge test, especially if no BWs and CEBs can be conducted. The US EPA, to quantify a membrane removal credit, suggests testing the membrane is done at its maximum nominal flow. This test is done only once in the membrane lifetime, so more sever conditions can be applied. ETV trials showed that the MS-2 LRVs were inversely proportional to the flux applied. However, within the same study, it was also observed that the LRVs for the lower flow rates were all within the range of the LRVs from the maximum flux test. Consequently, in the protocol developed in this study, the selection of flow rates below the maximum nominal values has been considered appropriate.

Feed and permeate samples are taken simultaneously at a given time, three times along the test (e.g. 5, 10, 15 min, according to the autonomy of the testing system). Several process parameters are monitored during the test, such as turbidity, conductivity, pH, temperature and transmembrane pressure (TMP). After the test, the system is drained, cleaned and filled with the water source normally treated (raw surface water), and a CEB is performed to ensure the removal of the surrogates.

Since the water used for the tests is tap water, there is no significant fouling is being deposited

during the test performance, and hence, the effect of adsorption to physically removable fouling or cake layer formation is minimised. Therefore, surrogates removal is ensured to be due to size exclusion, membrane adsorption and non-chemically removable fouling, but not due to particulate material either in suspension or deposited onto the membrane. According to Lozier et al. [20], integrity defects on the order of 200 μm can be masked by foulants, improving pathogen rejection. To determine if fouling has occurred during the test and if so, its impact in the surrogates removal rate, the LRVs and the membranes resistance along time have been plotted and no differences have been encountered (data not shown). This is in accordance with Martí et al. [21], who undertook challenge tests with virus surrogates in membrane bioreactors.

PDTs have been conducted just after the initial CEB performed, when potential membrane defects are uncovered, to compare both results. During the PDT, the membrane module is drained, compressed air (below the bubble point and above the pressure needed to determine a given defect) is supplied to the drained side of the wetted membrane and the pressure is maintained for a given time. In case of membrane integrity failure, the airflow through the pores will be orders of magnitude higher than the diffusion flow that would occur if the membrane integrity was not damaged. By measuring the pressure drop, the membrane integrity can thus be determined.

3. Results and discussion

3.1. Bacillus spores tests

Fig. 2 shows the LRV obtained for the four challenge tests conducted in the three membrane modules as well as the spiked concentration (C_f) in each case. As can be seen, the LRVs averages range from >3.9 to 5.2, indicating high removal. The SWTR [6] required 3 log removal of *Giardia*, which is slightly larger in size than *Cryptosporidium*, for the whole water treatment. Taking into account that the latter is more difficult to eliminate than *Giardia* [22], based on the results obtained, the membrane treatment itself is capable of removing at least approximately 1 more log than the established threshold for *Giardia*. As a result, the membranes alone would enable the fulfilment of this rule. The LT2ESWTR [9], based on the incoming water quality, requires different removal credits for *Crypposporidium*, ranging from 0 to 5.5 LRV for the whole treatment process. Considering the values obtained, the tested membranes would only require 1.5 more log removal to fulfil the most stringent requirement for the whole treatment process, which would typically be achieved with coagulation/flocculation/sedimentation [23], or coagulation itself [24] or to a larger extent by UV disinfection [25].

In the case of membrane A, differences found in the various tests conducted (Fig. 2 black bars) have not been significant since their average LRVs values have been 4.5, >4.4, >4.9, 4.8, so they differ in less

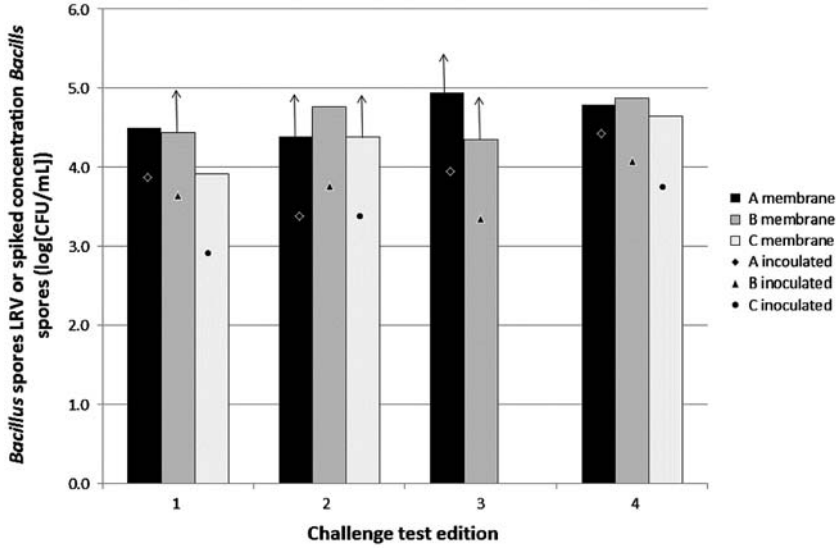

Fig. 2. LRVs and spiked concentration of *Bacillus* spores of the three membrane modules tested in the four challenge test trials performed. Black bars correspond to A membrane, grey ones to B membrane and white ones to C membrane. Diamond, triangle and circle symbols correspond to the spiked concentration in each test to A–C membranes, respectively.

than 0.5 LRV. However, it is important to remark that, in the second and third challenge test, the permeate concentration has been below the detection limit. Therefore, greater removal rates may have been obtained. Membrane B has also performed nearly constant along the various tests, presenting average LRVs values of 4.4, 4.8, >4.3, 4.9, which also vary in 0.5 logarithmic units (Fig. 2 grey bars). In the penultimate test, *Bacillus* spores permeate concentration has been below the detection limit. Thus, a greater removal rate may have been achieved. Membrane C has increased the LRVs obtained, being their average values >3.9, >4.4 and 4.6 (Fig. 2 white bars). Nonetheless, *Bacillus* spores concentrations in the permeate have been below the detection limit in the first two tests. As a result, the seeding stock concentration has been increased accordingly (3.0, 3.4 and 3.8 logs) in order to enable a precise quantification. This explains the increase in LRVs between tests concerning the membrane *C*.

It is important to highlight that, due to the fact that the test objective is to evaluate the suitability of the developed integrity protocol in the three UF configurations, the different membranes results should not be compared directly since their characteristics as well as the operational conditions applied have been different.

Theoretical LRV (LRV_t) for *Bacillus* spores based on the LT2ESWTR equation Eq. (2) differ from the real values obtained between 0.5 and 1.0 logarithmic units (15–20% in absolute terms). This difference can be attributed to the conservational approach taken when calculating it from a theoretical point of view, leading to lower removal values than the real ones. Nonetheless, the error obtained appears as acceptable especially if conducted as a routine test complemented by other more-accurate procedures that enable the detection of defects below 1–3 μm.

3.2. Bacteriophages tests

The characteristics of the bacteriophages enabled both the testing of membrane integrity and their capacity to remove enteric virus surrogates. Because of the size of *PDR-1*, around 60 nm, it should be removed by all the membrane systems tested due to size exclusion phenomena. The presence of this phage in the permeate streams may indicate that the membrane fibres are compromised. Fig. 3 shows the LRVs of the different membranes tested, which have ranged between 3.6 and 7.1 log, as well as the PDR-1 spiked concentration.

For all membranes, in all the editions except the second for membrane A and in all except the first for membrane C, the PDR-1 concentration in the permeate has been below the detection limit. However, the seeded concentration in the third test has been an order of magnitude lower than in the previous cases, which explains the lower LRVs values obtained. In the fourth edition, the detection limit has been decreased in 2 logarithmic units due to analytical issues, and similarly does the LRV obtained.

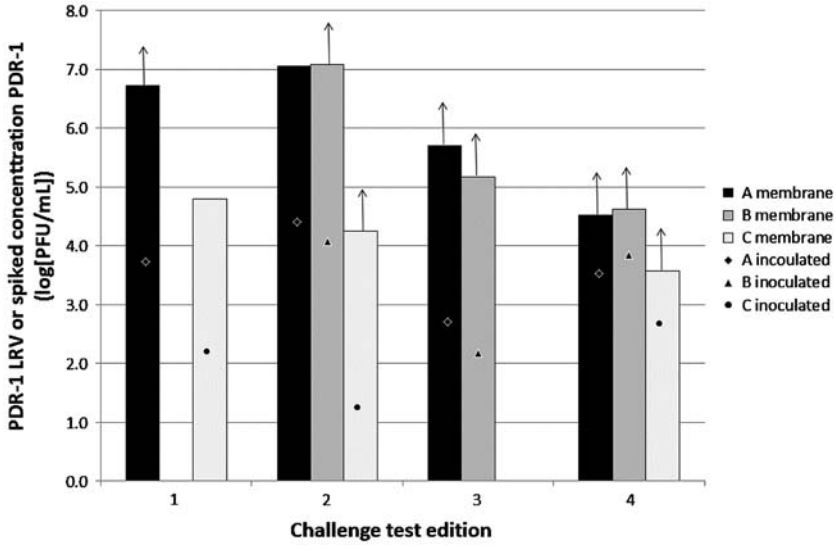

Fig. 3. LRVs and spiked concentration of PDR-1 of the three membrane modules tested in the four challenge test trials performed. Black bars correspond to A membrane, grey ones to B membrane and white ones to C membrane. Diamond, triangle and circle symbols correspond to the spiked concentration in each test to A–C membranes, respectively.

Despite the apparent decrease in the PDR-1 LRVs experienced, it is remarkable the fact that LRVs are quite high, suggesting that the membranes have been intact during the tested period (one year approximately). As pointed out previously, the direct comparison between membranes may not be adequate due to the differences in the seeding concentration (even though they are supposed to be minimised), the operational conditions tested during their lifetime, etc.

MS-2 and *GA* present smaller dimensions than the previous surrogates and are in the range of, or even slightly minor than, the membrane pore sizes tested. Consequently, their removal rates are expected to be lower, and they should not be taken as straightforward indicators of membrane integrity but as an evocation of changes in the membrane properties, which could preclude future damages.

Figs. 4 and 5 represent the LRVs per MS-2 and GA, respectively, for the three tested membranes as well as their spiked concentration. Due to operational issues, membrane C could not be tested in the second and third edition.

MS-2 has been used in previous study to assess membrane integrity [26–31], and it has been considered as a "worst case scenario" in terms of virus removal [32].

As can be seen, in this case, the average LRVs fluctuate to a larger extent than with the previous surrogates: in the case of membrane A2.1, 1.4, 2.4 and 1.8 LRVs (Fig. 4, black bars); for membrane B 3.2, 2.2, 1.3 and 2.8 LRVs (Fig. 4, grey bars) and for membrane C 5.6, 2.7 LRVs (Fig. 4, white bars). This may be explained by the main separation mechanism. With these smaller surrogates, adsorption and electrostatic interactions play a more significant role. As a result, the membrane fouling deposited (non-chemically removable) may lead to a greater surrogates retention. Consequently, the membrane resistance after the initial CEB divided by the virgin membrane resistance has been taken into account in all the experiments to identify potential differences originated from remaining fouling. Martí et al. [21] found that viral indicators LRV depended on irreversible fouling (not removable either by physical and chemical means), expressed by TMP, accumulated in the membrane. Nonetheless, the results obtained up to date do not show a clear relationship between the resistance when starting the experiment and the virgin membrane resistance with the LRV obtained. However, since the experiments have taken place at different seasons (i.e. water quality), fouling composition deposited on the membrane may be different and thus, present a different behaviour, enhancing or not removal rates. Besides fouling nature, the membrane properties themselves (hydrophobicity and electrical charge mainly), both virgin and after continuous operation, may also be responsible for this variability.

Regarding membrane C, the large LRVs differences encountered can be attributed to a larger particle content in the water used for the first trial (a turbidity increase was experienced in the water where viruses were spiked). This may had enhanced the association

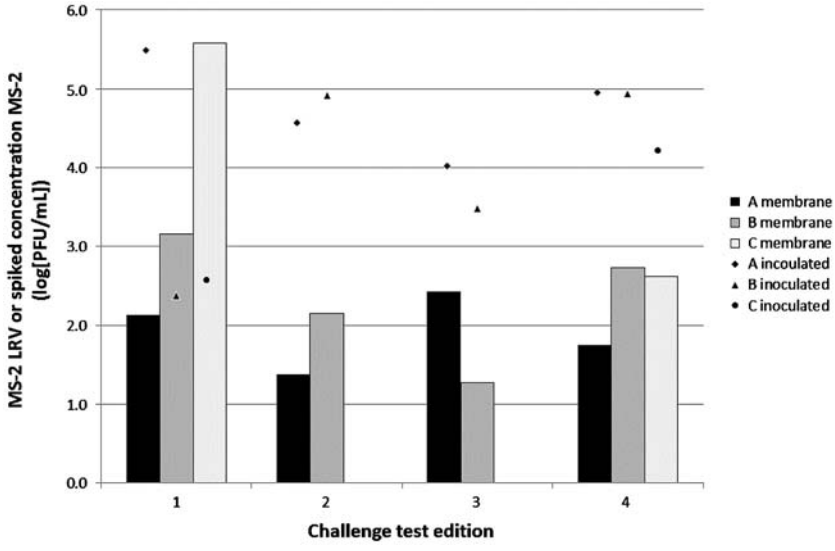

Fig. 4. LRVs and spiked concentration of MS-2 of the three membrane modules tested in the four challenge test trials performed. Black bars correspond to A membrane, grey ones to B membrane and white ones to C membrane. Diamond, triangle and circle symbols correspond to the spiked concentration in each test to A–C membranes, respectively.

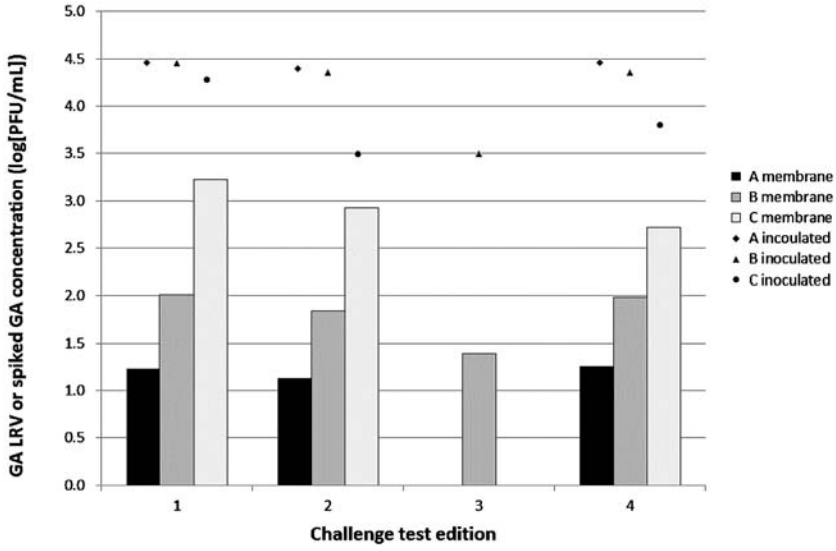

Fig. 5. LRVs and spiked concentration of GA of the three membrane modules tested in the four challenge test trials performed. Black bars correspond to A membrane, grey ones to B membrane and white ones to C membrane. Diamond, triangle and circle symbols correspond to the spiked concentration in each test to A–C membranes, respectively.

of bacteriophages [33], becoming more easily retainable by the membrane and hence, over estimating their removal rates.

Even though LRVs obtained are significantly lower than the 4 logs recommended by the USEPA for the whole treatment [6], it is important to take into account that the size of these bacteriophages is normally smaller than UF membranes pore size, so their removal only by size exclusion is not feasible. DWTPs based on membranes would encompass at least a final disinfection step, which is able to inactivate viruses in an easier way than other organisms like oocysts, which are highly resistant to chlorine and chloramines [5]. In this sense, the *Bacillus* spores testing previously described would ensure the system removal capacity of this protozoan parasite and the final disinfection stage the viruses.

Fig. 5 represents the LRV of each challenge test concerning GA and their spiked concentration. Membrane A average LRVs have been 1.2, 1.1, 1.3 (black bars), B membrane 2.0, 1.8, 1.4, 2.0 LRVs (grey bars) and 3.2, 2.9, 2.7 LRVs for C membrane (white bars). Analogously to MS-2 due to the separation mechanism nature, further factors such as deposited fouling and membrane characteristics may explain the fluctuation. Nevertheless, the variability has been smaller than in the previous case.

As can be seen MS-2 removal rates have been generally higher than GA ones, which is in accordance with previous studies [32]. This may be explained by the fact that the latter presents a greater hydrophobicity and thus has a greater affinity to be adsorbed onto and transported through the membrane, leading to lower LRVs. It has to be noticed that electrostatic interactions can also play a role in the removal mechanism of these surrogates; however, as surrogates zeta potential versus pH curve is not available, the importance of this phenomenon cannot be quantified.

3.3. Comparison of PDT and surrogates challenge tests

The PDTs performed before each challenge test in the three UF membranes have not detected any integrity failure during the four editions conducted (i.e. pressure drop experienced has been lower than the threshold which alerts of potential fibres impairment, which is defined by membrane manufacturers).

As commented previously, some differences have been found between the results obtained from the pressure decay values (LRV_t) and the surrogates inoculation and removal quantification by analyzing feed and permeate streams (LRV) in this work (0.5–1.0 log units). Qualitative PDT, based on checking if the pressure decay during the test is below a certain value indicated by the membrane manufacturer, is also indicative of the system integrity. However, it is more limited since theoretical LRVs cannot be calculated (through Eq. (2)). The most limiting feature of relying on PDT is the fact that defects smaller than 1 μm require pressures that cannot be held by low-pressure membranes. Therefore, a certain defect size is required to be noticed in terms of pressure decay, which may

mask impairments. Also, PDT accuracy is limited, since system failures (e.g. in valves and seals which do not necessary involve a permeate pollution) may result in false positives. On the other hand, PDT appears as an easy procedure to be implemented, which typically lasts less than 10–15 min, making it very attractive for water utilities when compared with other integrity tests. It has to be kept in mind that, in spite of its attractiveness, PDT requires stopping the production process.

The proposed challenge test with bacteriophages, especially PDR-1, and spores enables a reliable assessment of the membrane integrity. Nonetheless, it requires the system to temporally shutdown (typically 2–3 h) and to ensure a subsequent cleaning stage (even though the tested organisms are innocuous to human health). In order to avoid surrogates aggregation, it is important to keep the spiked concentration within certain ranges, which may limit the maximum LRVs to be verified. Results can be obtained in 24 h approximately and the associated analytical cost is low. Nonetheless, in the case of a DWTP dealing with feed water of relatively constant quality (pH, ionic strength) and low turbidity values, the bacteriophages could be spiked directly in the feed water (if allowed) and, thus, avoid the system to stop the process. Similarly, in case the bacteriophages load in the feed water would be high, the seeding step could be eluded, avoiding the necessity to stop the production process.

4. Conclusions

The developed protocol has been implemented successfully in the three existing UF configurations, enabling the performance of the test periodically.

PDR-1 and *Bacillus* spores rejections have been above 4 logs. This indicates that the fibres of the three modules assessed are not impaired (from an integrity standpoint), because the surrogates sizes are above the pore sizes assessed. These results are in accordance with the PDT outcomes, which enables the detection of defects in the range of 1–3 μm with the applied pressures (1–2 bar). In the case of MS-2 and GA retention, differences have been found between the different membranes and within the same one over time. In particular, MS-2 has typically been removed in a larger extent. Probably this is due to the dissimilarities in hydrophobicity (GA > MS-2) and isoelectric point (MS-2 = 3.1–3.9; GA = 2.1–2.3 depending on the ionic strength of the suspension solution) [14]. In this case, when the surrogates sizes are smaller than the membranes pore size, adsorption processes and electrostatic interactions play a significant role in viruses retention. As a result, the effect of fouling in the membrane as well as the membrane characteristics themselves, may explain the differences found.

Taking into account the data obtained nowadays, the use of PDR-1 appears as a suitable micro-organism to assess membrane integrity because its size is slightly above the UF tested membranes pore sizes (20–40 nm larger), and *Bacillus* spores as a control (around 1 μm larger). As a result, the removal mechanism of these two surrogates relies on size exclusion, so that fibre impairment will lead to a lower retention. MS-2 and GA removals, due to their smaller sizes, mainly rely on adsorption processes and electrostatic interactions, so that they may be influenced by further factors, such as non-chemically removable fouling of the membrane, feed water characteristics, etc., which may imply a more difficult data interpretation. Nevertheless, this data can be used to comprehend membranes performance, besides the potential of providing membrane integrity information. Consequently, more challenge tests with the same membranes will be conducted to obtain further conclusions in this direction.

The methodology proposed, with the three bacteriophages and *Bacillus* spores, can provide the operator not only with tools to determine the system integrity but also with information about the membrane status and hence contribute to the understanding of its behaviour and enable the adaptation of the operational conditions. The implementation of this methodology could be beneficial to award removal credits to membrane-based technologies, to undertake the direct testing in DWTP facilities and also in pilot testing studies, where pilot plants are exposed to extreme conditions, and hence, the validation of their removal pathogens rejection along time may be of utmost importance.

Acknowledgements

This work has been conducted under the financial support of the LIFE+ Programme of the European Commission within the framework of the UFTEC Project (LIFE09 ENV/ES/000467 UFTEC). The authors gratefully acknowledge Xavier Serrallach (CETaqua), Verónica García-Molina (Dow Chemical Ibérica) and BastiaanBlankert (Pentair X-Flow) for their technical contributions in this work.

Symbols

BW	—	backwash
CEB	—	chemically enhanced backwash
CFU	—	colony-forming unit
DWTP	—	drinking water treatment plant

ETV	—	environmental technology verification
IESWTR	—	interim enhance surface water treatment rule
IOS	—	international organization for standardization
LRV	—	logarithmic removal value
LRV	—	Logarithmic removal value (theoretical)
LT1ESWTR	—	long term 1 enhanced surface water treatment rule
LT2ESWTR	—	long term 2 enhanced surface water treatment rule
MF	—	microfiltration
UF	—	ultrafiltration
PDT	—	pressure decay test
PFU	—	plaque forming unit
Q_{breach}	—	Breach flow [L/min]
Q_p	—	permeate flow [L/min]
SWTR	—	surface water treatment rule
TMP	—	transmembrane pressure
US EPA	—	United Stated environmental protection agency
VCF	—	volumetric concentration factor
WHO	—	world health organisation

References

[1] H. Guo, Y. Wyart, J. Perot, F. Nauleau, P. Moulin, Low-pressure membrane integrity tests for drinking water treatment: A review, Water Res. 44 (2010) 41–57.

[2] R. Gimbel, Anforderungenan die Membrantechnik in der Trinkwasseraufbereitung. 5. AachenerTagung. Siedlungswasserwirtschaft und Verfahrenstechnik. Membrantechnik in der Wasseraufbereitung und Abwasserbehandlung. 3–921955-28-9, Aachen, 2003.

[3] H. David, P.E. Furukawa, E. Ch, NWRI Final Project Report: A Global Perspective of Low Pressure Membranes, USA, California, March, 2008.

[4] E. Zondervan, A. Zwijinenburg, B. Roffel, Statistical analysis of data from accelerated aging tests of PES UF membranes, J. Membr. Sci. 300 (2007) 111–116.

[5] US EPA, Membrane filtration guidance manual, 2003. Available on: http://www.epa.gov/ogwdw/disinfection/lt2/pdfs/guide_lt2_membranefiltration_final.pdf (accessed on July 2012).

[6] US EPA Surface Water Treatment Rule, 1986. Available on: http://water.epa.gov/lawsregs/rulesregs/sdwa/swtr/index.cfm (accessed on July 2012).

[7] US EPA Interim Enhanced Surface Water Treatment Rule, 1998. Available on: http://water.epa.gov/lawsregs/rulesregs/sdwa/ieswtr/ (accessed on July 2012).

[8] US EPA Long Term 1 Enhanced Surface Water Treatment Rule, 2002. Available on: http://water.epa.gov/lawsregs/rulesregs/sdwa/mdbp/lt1/lt1eswtr.cfm (accessed on July 2012).

[9] US EPA Long Term 2 Enhanced Surface Water Treatment Rule, 2006. Available on: http://water.epa.gov/lawsregs/rulesregs/sdwa/lt2/index.cfm (accessed on July 2012).

[10] C.P. Chauret, C.Z. Radziminski, M. Lepuil, R. Creason, R.C. Andrews, Chlorine dioxide inactivation of cryptosporidium parvumoocysts and bacterial spore indicators, Appl. Environ. Microbiol. 67 (2001) 2993–3001.

[11] E.C. Nieminski, W.D. Bellamy, L.R. Moss, Using surrogates to improve plant performance, J. Am Water Works Assoc. 92 (2000) 67–78.

[12] C. Radziminski, L. Ballantyne, J. Hodson, R. Creason, R.C. Andrews, C. Chauret, Disinfection of *Bacillus* subtilis spores with chlorine dioxide: A bench-scale and pilot-scale study, Water Res. 36 (2002) 1629–1639.

[13] J. Langlet, F. Gaboriaud, J.L. Duval, C. Gantzer, Aggregation and surface properties of F-specific RNA phages: Implication for membrane filtration processes, Water Res. 42 (2008) 2769–2777.

[14] M.H. Adams, Bacteriophages, Interscience Publishers Inc, New York, NY, 1959.

[15] International Organization for Standardization, ISO 10705–1: Water Quality—Detection and Enumeration of Bacteriophages. Part 1: Enumeration of FSpecific RNA Bacteriophages. International Organization for Standardization, Geneva, Switzerland, 1995.

[16] International Organization for Standardization, ISO 10705–2: Water Quality—Detection and Enumeration of Bacteriophages. Part 2: Enumeration of Somatic Coliphages. International Organization for Standardization, Geneva, Switzerland, 2000.

[17] Y. Matsui, T. Matsushita, S. Sakuma, T. Gojo, T. Mamiya, H. Suzuoki, T. Inoue, Virus inactivation in aluminium and polyaluminium coagulant, Environ. Sci. Technol. 37 (2003) 5175–5180.

[18] N. Shirasaki, T. Matsushita, Y. Matsui, M. Kobuke, K. Ohno, Comparison of removal performance of two surrogates for pathogenic waterborne viruses, bacteriophage Qβ and MS2, in a coagulation–ceramic microfiltration system, J. Membr. Sci. 326 (2009) 564–571.

[19] N. Shirasaki, T. Matsushita, Y. Matsui, T. Urasaki, K. Ohno, Comparison of behaviours of two surrogates for pathogenic waterborne viruses, bacteriophages Qβ and MS2, during the aluminium coagulation process, Water Res. 43 (2009) 605–612.

[20] J. Lozier, C. Colvin, J.H. Kim, M. Kitis, B. Mariñas, B. Mi, Microbial Removal and Integrity Monitoring of High-pressure Membranes, American Water Works Association Research Foundation, Denver, CO, 2003.

[21] E. Martí, H. Monclús, J. Jofre, I. Rodriguez-Roda, J. Comas, J.L. Balcázar, Removal of microbial indicators from municipal wastewater by a membrane bioreactor (MBR), Bioresour. Technol. 102 (2011) 5004–5009.

[22] Health Canada, Guidelines for Canadian Drinking Water Quality, 2010. Available on: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/protozoa/chap_8-eng.php (accessed on July 2012).

[23] WHO, Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water, 2004. Available on: http://www.who.int/water_sanitation_health/dwq/watreatment/en/index.html (accessed on August 2012).

[24] S.H. Lee, C.H. Lee, Y.H. Kim, J.H. Do, S.H. Kim, Occurrence of Cryptosporidium oocysts and Giardia cysts in the Nakdong River and their removal during water treatment, J. Water Health 51 (2007) 163–169.

[25] US EPA, Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule, 2006. Available on: http://www.epa.gov/ogwdw/disinfection/lt2/pdfs/guide_lt2_uvguidance.pdf (accessed on August 2012).

[26] J.G. Jancangelo, M. Watson R.R. Trusell, Role of membrane technology in drinking water treatment in the United States, Desalination 113 (1997) 119–127.

[27] J.C. Kruithof, J.C. Schippers, P.C. Kamp, H.C. Folmer, J.A.H.M. Hoffman, Integrated multi-objective membrane systems for surface water treatment: Pretreatment of reverse osmosis by conventional treatment and ultrafiltration, Desalination 117 (1998) 37–48.
[28] J. Langlet, L. Ogorzaly, J.C. Schrotter, C. Machinal, F. Gaboriaud, J.L. Duval, C. Gantzer, Efficiency of MS2 phage and Qβ phage removal by membrane filtration in water treatment: Applicability of real-time RT–PCR method, J. Membr. Sci. 326 (2009) 111–116.
[29] B.S. Oh, H.Y. Jang, Y.K. Jung, J.W. Kang, Micrifiltration of MS2 bacteriophage: Effects of ozone on membrane fouling, J. Membr. Sci. 306 (2007) 244–252.
[30] B. Zhu, D.A. Clifford, S. Chellam, Virus removal by iron coagulation–microfiltration, Water Res. 39 (2005) 5153–5161.
[31] K. Zodrow, L. Brunet, S. Mahendra, D. Li, A. Zhang, Q. Li, P.J.J. Alvarez, Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal, Water Res. 43 (2009) 715–723.
[32] N. Boudaud, C. Machinal, F. David, A. Fréval-Le Bourdonnec, J. Jossent, F. Bakanga, C. Arnal, M.P. Jaffrezic, S. Oberti, C. Gantzer, Removal of MS2, Qβ and GA bacteriophages during drinking water treatment at pilot scale, Water Res. 46 (2012) 2651–2664.
[33] M.R. Templeton, R.C. Andrews, R. Hofman, Removal of particle-associated bacteriophages by dualmedia filtration at different filter cycle stages and impacts on subsequent UV disinfection, Water Res. 41 (2007) 2393–2406.