Virus removal retention challenge tests performed at lab scale and pilot scale during operation of membrane units

H. Humbert, C. Machinal, Ivan Labaye and J. C. Schrotter

ABSTRACT

The determination of the virus retention capabilities of UF units during operation is essential for the operators of drinking water treatment facilities in order to guarantee an efficient and stable removal of viruses through time. In previous studies, an effective method (MS2-phage challenge tests) was developed by the Water Research Center of Veolia Environnement for the measurement of the virus retention rates (Log Removal Rate, LRV) of commercially available hollow fiber membranes at lab scale. In the present work, the protocol for monitoring membrane performance was transferred from lab scale to pilot scale. Membrane performances were evaluated during pilot trial and compared to the results obtained at lab scale with fibers taken from the pilot plant modules. PFU culture method was compared to RT-PCR method for the calculation of LRV in both cases. Preliminary tests at lab scale showed that both methods can be used interchangeably. For tests conducted on virgin membrane, a good consistency was observed between lab and pilot scale results with the two analytical methods used. This work intends to show that a reliable determination of the membranes performances based on RT-PCR analytical method can be achieved during the operation of the UF units.

Key words | membrane, meal time RT-PCR, virus challenge test, virus removal

INTRODUCTION

Membrane technology has emerged, in the last 20 years, as one of the main contributors to solve water problems. Despite a fast growing market, membrane technology still has to reach the level of application of many competing water treatment processes. There is currently a need for precisely characterising membranes in relation to their application in water filtration.

In the last decade, protocols and tools for membrane characterisation were developed by the scientific community, organisations and companies (Jacangelo & Adham 1995; USEPA 2001; Jacangelo et al. 2002, 2005; Capannelli et al. 2004; Machinal et al. 2006).

MS2 phage, as a surrogate, is used to evaluate pathogenic virus removal (Jacangelo & Adham 1995; Ogorzaly & Gantzner 2006; Langlet et al. 2008; Langlet et al. 2009). Until now phage removal was commonly defined upon comparing the numbers of infectious units in permeate with that in feed solutions, i.e. with PFU culture analytical methods (PFU, Plaque Forming Units).

Veolia Environnement, has selected the RT-PCR (Reverse Transcription Polymerase Chain Reaction) analytical methods in order to improve the previous control in terms of rapidity, sensitivity or specificity, based on a molecular analytical method and a protocol developed by Ogorzaly & Gantzner (2006).

At lab scale, the objective of this study was to validate the use of RT-PCR as an analytical method for determining the log removal value (LRV) in the MS2phage challenge test on membranes.

At pilot-scale, the objective was to compare with lab-scale experiment and to follow-up membrane performance in operation. The aim was to determine virus retention capabilities of UF units during operation with standardized and molecular biology methods.

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MATERIALS AND METHODS

MS2 detection methods: RT-PCR and PFU methods

MS2 phage is currently used as a surrogate to evaluate pathogenic virus removal efficiency by filtration membrane during water treatment (Jacangelo & Adham 1995). The MS2 phage is small (≈25 nm diameter), and has a low isoelectric point (pI = 3.5–3.9) which indicates a significant negative charge carried by the virus at neutral pH conditions (Langlet et al. 2008; Ogorzaly et al. 2007).

Virus removal by membrane filtration is estimated from the aqueous phase using two analytical methods: 1) culture detection using standardized methods and quantification of plaque forming units corresponding to infectious bacteriophages (ISO Norm 10705-1) and 2) molecular biology method detection of F-specific RNA bacteriophage genome i.e. reverse transcription (RT)-real time PCR. (Ogorzaly & Gantzer 2006).

The culture of bacteriophages allows the detection of infectious particles. However, RT-PCR targets the bacteriophage genome (RNA) and detects all viral particles regardless of their infectivity. The Table 1 gives a comparison of both analytical methods.

Estimation of the initial virus load and viral removal

The same volumes of each membrane permeate and spiked solutions were analysed in duplicates by culture (1 ml) and by RT-PCR (140 μl).

The viral removal by the membrane was determined according to the following formulæ:

- using RT-PCR detection:

\[ LRV_{RT\_PCR} = \frac{C_{\text{permeate}} - C_{\text{feed}}}{3.32} \]

With: - LRV: Log Removal Value
- \( C_{\text{permeate}} \): membrane permeate Ct mean value
- \( C_{\text{feed}} \): spiked solution Ct mean value

- using PFU culture detection:

\[ LRV_{PFU} = \log_{10} \left( \frac{[\text{MS2 in the feed}]}{[\text{MS2 in the permeate}]} \right) \]

With: - LRV: Log Removal Value
- [MS2]: concentration in PFU/mL

Concerning the determination of the factor (3.32) in the formula, Ten fold dilutions of F-RNA phage stocks (10^{-2} - 10^{5} PFU/ml) were made with PBS 0.1 mM pH 7.2 in order to construct the standard curves. The dilutions were analysed by double agar layer plaque assay method to define concentrations in PFU/ml. Positive and negative controls were added in each assay. The Ct values of each dilution amplified in triplicate by RT-real time PCR were plotted as a function of logarithm of starting quantity of phages (PFU/ml).

A linear response was observed from bacteriophage's concentration between 10^{5} to 10^{-1} PFU/ml. The slope of the standard curves obtained was between –3.24 and –3.58 with a squared correlation coefficient \( R^2 > 0.98 \). The factor 3.32 corresponds to the slope of the calibration curve in the optimal conditions.

Table 1 | Comparison of both analytical methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFU</td>
<td>– Detect infectious i.e.&quot;active&quot; viruses lysing bacteria</td>
<td>– Overestimation of LRV possible if viruses are inactivated or/and aggregated after filtration</td>
</tr>
<tr>
<td></td>
<td>– Easy to use and rapid compared to PFU</td>
<td>– Need of a precise protocol to prevent aggregation</td>
</tr>
<tr>
<td></td>
<td>– Detecting all viruses (active, inactive, aggregated, etc.)</td>
<td>– Time consumption</td>
</tr>
<tr>
<td></td>
<td>– Detect free RNA</td>
<td>– Detecting all viruses (active, inactive, aggregated, etc.)</td>
</tr>
<tr>
<td></td>
<td>– Underestimation of LRV possible if a small amount of broken viruses are generated during the filtration</td>
<td>– Need to control the purification level of virus stocks and to control the process conditions to prevent virus degradation</td>
</tr>
</tbody>
</table>
Membranes tested at lab scale

Thirteen virgin membranes were selected for estimating their virus removal efficiencies in bench-scale experiments: three microfiltration membranes and ten ultrafiltration membranes. Membranes tested were hollow fibers assembled in modules or flat sheet membranes introduced in a flat sheet cell.

Lab scale challenge test protocol

In previous studies, optimal operating conditions have been tested. (Overby et al. 1966; Ogorzaly & Gantzer 2006; Langlet et al. 2008; Machinal et al. 2009).

Bacteriophage removal by the membranes was monitored on a laboratory-scale unit. A volume of viral suspension with a known amount of MS2 phage (up to $10^6$ PFU/mL) was prepared in 0.2 mM Phosphate Buffer Saline (PBS) solution at neutral pH. Three identical modules (triplicate) were placed in parallel in the filtration apparatus. Filtrations were carried out under constant pressure in frontal mode, at ambient temperature ($22 \pm 2^\circ C$) and for a variable time depending on the permeability of the tested membranes. Constant volumes of suspension were filtered per membrane. Samples of viral solutions were taken before (feed solutions) and after filtration (permeate), and were analysed by the molecular biology method (real-time RT-PCR) and culture method (PFU).

Membranes tested at pilot scale and pilot unit description

MS2 challenge tests were performed at pilot scale during the operation of different pilot units. These pilot units are all installed at the same location near a drinking water plant (DWTP) in France. They have capacities ranging from 5 to 10 m$^3$/h and were operated under various conditions and/or types of water (raw, settled, filtered water) depending on the main application studied. Each pilot unit was equipped with a specific type of module.

The results collected to date mainly originated from three different types of modules installed on three different types of pilot unit: module A, module B and module C. These modules are dedicated to drinking water production and are all considered as UF membranes by their respective supplier. Module C and B correspond to modules already available on market. Module An is a module under development. Table 2 summarizes the main characteristics of these three modules. In addition to the results obtained on modules A to C, some results were also obtained with other types of modules (UF or MF, pressurized or immersed, available on market).

Pilot scale challenge test protocol

Water quality used during the test

With regard to tests conducted at lab scale, particular attention must be paid to feed water used during the MS2 challenge tests. Water quality has to satisfy certain specifications (low suspended solids (SS), low chlorine concentrations) in order to avoid any disturbing phenomenon such as cell aggregation or cell damage that can modify the results. In our case, preliminary tests and analyses had showed that sand filtered water produced by a near-by DWTP was appropriate for such kind of test.

Cleaning of the pilot unit

Before each test, the pilot unit was stopped and cleaned for a few hours with the selected water (sand filtered water) in order to eliminate any suspended solids, particles or chemicals from the tanks and the pipes that could affect the results.

Pilot operation during the test

Each test was operated under the same operating conditions (ie water flow and filtration/backwash cycle durations) as those applied during the trial.

Table 2 | Characteristics of the main UF modules studied at pilot scale

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Commercial/ development state</th>
<th>Operating conditions on the pilot unit (flux, recovery rate and SS concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Module A</td>
<td>UF submerged hollow fiber</td>
<td>Under development</td>
<td>Harsh conditions (out of the ranges specified by the supplier)</td>
</tr>
<tr>
<td>Module B</td>
<td>UF Pressurized</td>
<td>Available on market</td>
<td>In the ranges specified by the supplier</td>
</tr>
<tr>
<td>Module C</td>
<td>UF submerged hollow fiber</td>
<td>Available on market</td>
<td>In the ranges specified by the supplier</td>
</tr>
</tbody>
</table>
Water doping, sampling and analysis

Figure 1 depicts the device that was set up and used to perform the tests at pilot scale whatever the pilot unit studied. A solution of concentrated MS2 was first prepared with sand filtered water. This concentrated solution was then injected on-line and diluted in the main flow of feed water (5 to 10 m$^3$/h of sand filtered water depending on the pilot unit) to obtain a feed water doped with MS2 at a concentration of 10$^7$ PFU/mL. The injection was performed throughout the duration of a whole filtration cycle. Filtration cycle durations range from 15 to 30 min depending on the pilot unit. Three to six samples of concentrated MS2 solution, doped feed water and permeate were collected throughout the filtration time. Samples dedicated to cell culture analysis (PFU method) were collected in sterile 250 mL bottles (with 5 mg sodium thiosulfate). Samples dedicated to RT-PCR analysis were collected in sterile 50 mL bottles. All samples were conserved at 4°C and analysed within 12 to 24 hours.

Comparison of the results obtained at lab and pilot scale

From time to time and after the completion of the whole pilot scale protocol, the pilot unit was stopped, modules were extracted from membrane tanks and fibers were taken from the modules to compare the results obtained at pilot scale with those obtained at lab scale. The pilot modules were finally repaired according to the supplier’s recommendations.

After sampling, fibers were conserved in deionized water at 4°C before their evaluation at lab scale (cf the description of the corresponding protocol in a previous paragraph).

RESULTS AND DISCUSSION

Lab scale protocol: comparison of both analytical methods

MS2 phage concentrations were quantified before and after filtration by both PFU and real-time RT-PCR analytical methods, and the Log Removal Values (LRV-log10) were calculated with both analytical results obtained on an average of three samples for UF and MF virgin commercial membranes. Figure 2 presents the comparison of both LRV.

A good consistency was obtained between both analytical techniques (PFU and RT-PCR). These results were in agreement with previous results published (Langlet et al. 2009), and a linear relationship between real-time RT-PCR and PFU methods was observed ($n = 46$ for MS2 phage).

Pilot-scale protocol

It is reminded that MS2 challenge tests were performed during different pilot trials conducted on different pilot units equipped with different types of membrane and running under different operating conditions. Two types of analytical tool (PFU culture and RT-PCR) were investigated to evaluate the performances of the membranes toward MS2 rejection (LRV) for both lab-scale and pilot scale protocols. The main
objectives were 1) to compare the results obtained at lab and pilot-scale 2) compare the results obtained with the two analytical in both case (i.e. pilot and lab scale) and 3) evaluate the performances of different type of modules (operated in different conditions for different type of application) toward MS2 rejection throughout time at pilot scale.

**Comparison of lab and pilot scale protocol**

Figure 3 presents an example of LRV values obtained at pilot scale as a function of LRV values obtained at lab-scale from fibers taken from different types of module (UF or MF new modules) by using either PFU culture analysis (circle dot) or RT-PCR analysis (square dot). It is important to notice that work is still in progress in order to confirm the general tendencies observed to date.

These first data highlight that the results obtained at pilot scale were similar to those obtained at lab scale whatever the technical analysis studied. In other words both types of protocol (i.e. lab or pilot-scale) can be used independently for the evaluation of the performances of the membranes towards MS2 rejection throughout operation time. Such intermediary conclusion is of particular importance since lab scale protocol required more time and is more difficult to set up than pilot one. Indeed, fiber sampling is often a critical operation since module reparation is generally not so easy to perform. Moreover, specific skills for the construction of the mini-modules and a specific filtration device are required to perform the tests at lab-scale. In addition, a good consistency between lab-scale and pilot-scale unit is of particular interest to compare commercially available membranes: performing lab-scale screening tests would assess membrane material as well as pilot-unit performance.

**Comparison of the 2 analytical methods for challenge tests conducted at pilot scale**

Figure 4 compares the two analytical tools for the determination of the LRV values at pilot scale. The different LRV values originated from different types of module (UF or MF, either new or in operation).

As for lab scale protocol, a good consistency is observed between RT-PCR analysis and PFU cultures analysis from samples taken directly from the pilot unit. In other words results show that whatever the membrane studied (UF or MF, new or in operation) both types of analytical method can be implemented independently and that both of them provide similar results within the context of a protocol conducted at pilot scale.

**Example of the evolution of the MS2 rejection performances throughout operation time**

MS2 rejection performances were monitored at pilot scale all along the operation of module A (Figure 5). Module An is an immersed UF hollow fiber membrane that was operated under very harsh operating conditions (higher flux, higher recovery rates and higher SS concentrations than those recommended by the supplier). The module was operated without PAC (Powdered Activated Carbon) for two months and then with PAC for four additional months in order to accelerate the aging of the module.

Initial LRV values evaluated with RT-PCR or PFU culture has dropped from about 4 log to about 2 log after only 2 months of operation without PAC. After two, three and four additional months of operation in presence of PAC, LRV values obtained at pilot scale with both analytical tools were then all lower than 1 log.
As shown by the example depicted in Figure 5, monitoring the performances of MS2 rejection is of great importance since UF modules could lose their initial performances throughout time.

Comparison of the MS2 rejection performances of different modules throughout operation time

Figure 6 compares the initial and final (ie after 6 to 12 month operation) LRV values obtained at pilot scale for modules A, B and C. Module B and C are modules available on market that were operated within the ranges of conditions specified by the suppliers. Module A is a module that was operated under harsh conditions (higher flux, higher recovery rates and higher SS concentrations than those recommended by the supplier).

When operated as recommended by the supplier, the two modules B and C exhibited stable MS2 rejection performances throughout time where as too harsh conditions like those applied on module C seriously affect the performances of the membrane.

This study intends to show the great interest of such a pilot scale protocol, that is as reliable as the protocol conducted at lab scale but more easy to set up. In theory, operating a membrane within the ranges specified by the supplier should guarantee stable performances toward virus rejection. In practice, variation can occur and such a pilot protocol proves to be a new and promising solution in order to evaluate the performances toward MS2 rejection (i.e. virus rejection from a more general point of view) throughout operation time.

CONCLUSION

Since 2004, The Veolia R&D center has been working on the development of a unique expertise for characterizing, selecting and following-up membrane performance during operation. Among different tools, the virus challenge test is a powerful tool to assess membrane performance both at lab-scale and pilot-scale unit. Preliminary data show a good consistency between lab and pilot scale units. This result is of particular interest to compare commercially available membranes as it opens the way of performing lab-scale screening tests that would allow assessing pilot-unit virus removal rate in addition to membrane virus removal rate thus demonstrating that the process parameters in normal operating conditions have little impact on virus challenge tests. Nevertheless, further experiments need to be performed on different module configuration (outside-in / inside-out / submerged / pressurized) with different operating conditions.
(flux, pressure, temperature,…) to validate these first data. This paper has also demonstrated that a reliable determination of the membranes removal based on the RT-PCR analytical method can be achieved during the operation of the UF plant units.

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