Characterization of the inline virus removal performances in hollow fibre modules by a new tracer electrochemically detected

L. Soussan, M. Alquier, C. Guigui, S. Alfenore, S. Mathe and C. Cabassud

ABSTRACT

In a previous work, an innovative and patented method was presented, that is based on a new virus surrogate. This surrogate is an enzyme-labelled MS2 phage that is directly detectable and quantifiable by amperometry thanks to its induced enzymatic activity. In this work, this method was used to characterize the dynamics of virus removal in micromodules as a function of the membrane defect size and the transmembrane pressure (TMP). Experiments were performed at lab scale with an integer module and with modules made with the same fibres with calibrated holes (50, 100, 150 μm) made in one of the module fibres with the laser method. Results showed first that the new method allowed diagnosing a 50 μm hole on the fibre of a micromodule. It was also demonstrated that, whatever the applied TMP, removal performances are all the more better that the defect size is smaller. Moreover, whatever the hole size, a TMP step rise from 1 to 1.5 bar during tracer filtration led to no significant change in the observed removal but improved the intrinsic removal performances.

Key words | defect detection, dynamics of removal, membrane integrity, MS2 bacteriophage, virus removal, virus surrogate

INTRODUCTION

Ultrafiltration processes are efficient barriers to retain viruses and microorganisms. Their application in the field of water production and reuse have gained considerable interest since the 1990s because of their beneficial sanitary impact on the water quality. However, ageing, fouling or damaging of the membranes could lead to reductions in the performances of membrane systems in terms of disinfection. Most of the membrane-based drinking water plants are equipped with an air pressure test to monitor membrane integrity. Some plants are also requested to control the microbial removal of the modules by offline tests with MS2 phages, according to the ASTM test (Standard Practice for Integrity Testing of Water Filtration Membrane Systems, 2009). A current key issue is to control the membrane efficiency in terms of virus removal and to detect the risk of virus leakage during filtration. Many studies highlighted the influence of various filtration operating or input parameters at steady state on the global virus removal (i.e. measured at the end of the filtration) (Herath et al. 1999; Farahbakhsh & Smith 2004; Fiksdal & Leiknes 2006; Arkhangelsky & Gitis 2008; Langlet et al. 2009). However, there is a lack of knowledge on the dynamics of the virus removal related to variations of operating conditions, as for example during the gradual increase of transmembrane pressure when operating at constant flow.

An inline tool for the characterization of the virus removal would thus be interesting for water producers, membrane manufacturers and water-end users. Existing methods to quantify viruses or virus surrogates present limitations for the inline characterization of virus removal (Brussaard 2004; Los et al. 2005; Ovadia & Gitis 2006; Moulin 2007; Pontius et al. 2009; Guo et al. 2010; Ovadia & Gitis 2006).
Sivaganesan et al. (2010). A recent study on the development of decision aid tools (Brehant et al. 2010) pointed out that a challenge test with MS2 phages in perforated micromodules does not allow detection of holes smaller than 60–200 μm and that pressure drop is not measureable for holes lower than 60 μm. In a previous study, a new method based on the use of a new virus surrogate was developed and patented to characterize removal dynamics (Guigui et al. 2010). This new tracer was designed in order to be directly detected/quantified in the produced waters while remaining representative of native viruses in terms of biochemistry, size and shape (Soussan et al. 2011a, b). This virus surrogate is an MS2 bacteriophage modified on its surface by the grafting of enzymes that catalyze a rapid reaction enabling the detection/quantification of this tracer by amperometry. The principle of the methodology is detailed in Soussan et al. (2012).

A recent work demonstrated notably that this method can be used to distinguish an ultrafiltration and a microfiltration membrane by their performance to retain this tracer (Soussan et al. 2012).

The objective of the present study was to evaluate if this method can be used to detect defaults in membranes. The aim was also to study the virus removal and the possible effect of the transmembrane pressure on the removal dynamics, related to the default size. Today the tracers are not yet available in large quantities and are made in the laboratory. So, the first step before testing this method on semi-industrial or large-scale modules was to assess the methodology for lab-scale microprototypes. In this work the new method was implemented for the inline characterization of the removal performances of hollow fibre micromodules. The presence of membrane defects was studied by characterizing modules having calibrated holes of different sizes. The influence of a transmembrane pressure (TMP) increase on the removal dynamics was also assessed by applying a pressure step rise during filtration.

MATERIALS AND METHODS

Microprototypes and their characterization

Four different microprototypes were used for the study. They were all based on inside-out ultrafiltration hollow fibres (provided by Aquasource-France) made of the same material, cellulose acetate, with a 0.01 μm average pore size. These fibres had a 0.93 mm inner diameter and a 0.73 mm thickness. One micromodule contained fibres without defect and the three others (named, respectively, M50, M100 and M150 μm) showed a defect consisting of a calibrated hole (50, 100, 150 μm diameter) voluntary made in one of its fibres with the laser method. Each hole was sharp and penetrated the fibre surface on the opposite side to the hole. The modules were composed of bundles of 10 to 16 fibres that were introduced in a 30 cm length PVC carter with a 1.4 cm internal diameter and a 35.5 ml volume (Vpshell). Table 1 presents the main characteristics of the microprototypes. In this table, Jh/Jm represents the proportion of permeate flow rate in the hole (Jh) related to the total module permeate flow rate (Jm). Jh was obtained by using the Hagen–Poiseuille law, considering the hole as a cylindrical pore.

The initial permeabilities Lpo of the modules ‘Without defect’, ‘M50 μm’ and ‘M100 μm’ were quite similar and in the range of the permeability given by the membrane manufacturer (220–300 L h⁻¹ bar⁻¹ m⁻² at 20 °C) for this fibre. On the contrary, the ‘M150 μm’ module showed a higher permeability which can be explained by the fact that the

<table>
<thead>
<tr>
<th>Modules</th>
<th>Number of fibres (--)</th>
<th>Membrane area, S (cm²)</th>
<th>Lpo at 20 °C (L h⁻¹ bar⁻¹ m⁻²)</th>
<th>D – membrane area/hole area (--)</th>
<th>Jh/Jm – permeate flow in the hole/total module flow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without defect</td>
<td>10</td>
<td>69</td>
<td>281 ± 25</td>
<td>/</td>
<td>0</td>
</tr>
<tr>
<td>M50 μm</td>
<td>16</td>
<td>105</td>
<td>318 ± 29</td>
<td>5.35 × 10⁶</td>
<td>0.3</td>
</tr>
<tr>
<td>M100 μm</td>
<td>16</td>
<td>107</td>
<td>303 ± 27</td>
<td>1.36 × 10⁶</td>
<td>1.2</td>
</tr>
<tr>
<td>M150 μm</td>
<td>16</td>
<td>109</td>
<td>385 ± 35</td>
<td>6.17 × 10⁷</td>
<td>2.4</td>
</tr>
</tbody>
</table>
flow in the 150 μm hole was more than 2% of the global flow of the module.

**Production and detection of the virus surrogates**

Virus surrogates (tracers) were freshly prepared the day of the filtration according to the protocol described in Soussan et al. (2011a). Their concentration expressed in pfu equivalents was $4.5 \pm 0.1 \times 10^9$ pfu mL$^{-1}$. The tracer suspensions were prepared in a PBS buffer solution (0.1 M, pH = 7.0 ± 0.1). The procedure for the amperometric detection and the details on the equipment and reagents are given in Soussan et al. (2012).

**The filtration pilot plant**

Figure 1 shows the flow sheet of the lab-scale filtration pilot plant. The module was operated in dead-end mode and could be fed at a constant flow rate with a volumetric pump (PCM Moineau 0.03ID10 model).

The feed solution was stored in a 20 L tank. The feed solution (called PBS/2) was made with phosphate buffered saline (PBS) diluted twice in ultrapure water. The objective was to maintain the tracer in a liquid matrix that was similar to the one where the tracers were produced. The tracer suspension was stored in a specific tank and was injected at the module inlet at a constant flow rate by a peristaltic pump. $C_{\text{feed}}$ was the tracer concentration at the module inlet.

The permeate was collected and the total cumulated volume was weighted inline with an electronic balance. A pressure sensor and a temperature sensor allowed monitoring these parameters at the module inlet.

Flushes were performed by circulating the PBS/2 solution in the fibres (see Figure 1) at a velocity of 4.2 m s$^{-1}$. Backwashes at constant pressure (2.00 ± 0.25 bar) were also performed by pushing with compressed air a PBS/2 solution. The backwashed waters were collected at the inlet and at the outlet of the modules then mixed for analysis.

**Filtration protocol**

The tracer suspension was injected so as to obtain the same feed concentration $C_{\text{feed}}$ at the module inlet for all trials ($2.1 \pm 0.1 \times 10^8$ eq. pfu mL$^{-1}$), which is within the range of the concentrations traditionally used to characterize MS2 removal.

A first injection of the tracer was performed at a filtration pressure of 1 bar. Tracer was injected until a permeate volume...
of about 150 ml was reached. Then PBS/2 was filtered alone at 1 bar TMP to study the return to equilibrium of the modules (filtrated volume about 260 ml).

Then the pressure was increased to 1.5 bar TMP and a second tracer injection was performed when the permeate flow rate was stable. The same procedure as for the 1 bar TMP was then applied for the 1.5 bar TMP.

At the end of each experiment, flushes were performed. The volume of liquid collected after each flush was between 10 to 15 times the internal fibre volume. Backwashes were then achieved. The total volume collected after each backwash was between two to three times the permeate volume. The final permeability Lpf of the module was then measured with PBS/2. The membrane permeability was measured at different pressures and the permeability at 20 °C was calculated by considering the temperature correction factor due to the viscosity.

Sampling and analysis of the tracers

At different times i, samples of permeate corresponding to cumulated volume of permeate Vp,i were taken in glass beakers in order to limit adsorption phenomena. Amperometric characterizations were performed the day of the filtration experiment according to the procedure detailed in Soussan et al. (2012). Analyses were also performed on feed, concentrate, flush and backwash samples.

Analysis of the filtration performances

Two kinds of parameters were considered to characterize filtration performances: the permeability and the tracer transmission through the membrane. These parameters were studied as a function of the cumulated filtrated volume (Vp,i) related to the carter volume (Vp,shell), enabling characterization of the renewal rate of the cartridge.

Two parameters were used to quantify the tracer transmission:

- The observed transmission ratio, Tobs, which was obtained at time i from the tracer concentration in the permeate Cp,i and the tracer concentration Cfeed in the feed at the inlet of the module (which was constant) according to Equation (1):

\[ T_{\text{obs}} = \frac{C_{p,i}}{C_{\text{feed}}} \]  

(1)

This transmission ratio is commonly used to calculate the LRV (logarithmic removal value) but does not take into account the concentration effects due to virus removal by the membrane and the real concentration in the module is higher than Cfeed.

- The intrinsic transmission ratio Tint, which was defined in order to take into account the concentration of tracer in the module during batch filtration. Tint was defined as the ratio of the tracer quantity qP collected in the permeate at time i to the total tracer quantity accumulated in the fibres at time i, according to Equation (2):

\[ T_{\text{int}} = \frac{q_P}{(q_{\text{feed,cum}} - q_{P,cum})} \]  

(2)

where qP (eq. pfu) is the tracer quantity collected in the permeate at time i, qfeed,cum (eq. pfu) is the cumulated tracer quantity fed to the module at time i, and qP,cum (eq. pfu) is the cumulated tracer quantity collected in the permeate at time (i-1).

RESULTS AND DISCUSSION

Variation of permeability during the different steps

For each module, the permeability was nearly constant during the experiments and was not affected by the different steps of the procedure: injection of tracer or TMP enhancement. The permeability was close to the initial permeability Lpo for all the tested modules. No measurable effect of fouling was thus highlighted on the basis of the permeability criteria either for the integer module or the damaged modules. In the same way, no fouling of the defects could be thus observed as the holes were little involved in the water transfer at the scale of the modules. Holes had therefore little impact on the global fouling of the system (see the parameter Jh/Jm in Table 1).

The paper will now focus on the study of the tracer transmission through the membranes in relation to the different steps of the filtration.
Transmission of virus surrogates during injection/filtration at 1 bar TMP

Figure 2 shows the dynamics of the observed transmission rate $T_{\text{obs}}$ (Figure 2(a)) and the intrinsic transmission ratio $T_{\text{int}}$ (Figure 2(b)) obtained with the microprototypes during the first step of the experiments at a constant TMP of 1 bar.

Tracer transmission was observed till the beginning of the tracer filtration (Figures 2(a) and 2(b)) for all modules. A major result is that this method allows diagnosis of a hole of 50 μm on a fibre of a micromodule by comparison to the reference module without defect.

Moreover, the transmission was all the more important than the defect was large. The transmission was thus due to the presence of the defect and to the transfer of virus surrogates by convection in the defect with the water flow. This result is in accordance with the results of Pontius et al. (2009) and Brehant et al. (2010).

Finally, $T_{\text{int}}$ decreased significantly with the filtrated volume. This behaviour can be explained by the fact that the tracer quantity fed and concentrated in the fibres increased significantly during the filtration whereas the tracer quantity was broadly constant in the permeate (Figure 2(a)). The intrinsic removal performances of all modules were thus improving during the tracer filtration.

Figure 2 | Dynamics of the observed transmission rate $T_{\text{obs}}$ (Figure 2(a)) and dynamics of the intrinsic transmission rate $T_{\text{int}}$ (Figure 2(b)) obtained during the injection of tracers at 1 bar and during the PBS/2 filtration at 1 bar for the UF modules (with and without defect).
Analysis of the tracer transmission when the tracer injection was stopped

Immediately after stopping the injection of the tracer suspension, some tracers still pass through the defects (Figures 2(a) and 2(b)), which means that tracers are staying in the fibers at the end of the tracer injection and that a quite important volume of filtration with pure liquid is required before obtaining a negligible transmission of tracers.

Contrary to the M100 and M50 μm modules exhibiting a moderate decrease of the transmission rate, the transmission rate for the M150 μm module decreases sharply (to one third) and then continues to decrease significantly (Figures 2(a) and 2(b)). The difference in these dynamics seems logical and may be explained by the fact that the amount of tracer that remains in the fibers at the end of the tracer injection is lower when the defect is larger.

Effect of a TMP increase (to 1.5 bar) on the tracer transmission during their injection and after the injection

A TMP step (to 1.5 bar) was then applied to each module before injecting tracers again at 1.5 bar. Figure 3 shows the

![Graph](image_url)

**Figure 3** | Dynamics of the observed transmission rate $T_{obs}$ (Figure 3(a)) and dynamics of the intrinsic transmission rate $T_{int}$ (Figure 3(b)) obtained during the pressure step rise $\Delta$TMP (from 1 to 1.5 bar), during the second tracer injection at 1.5 bar and during the PBS/2 filtration at 1.5 bar for the different UF modules (with and without defect).
dynamics of $T_{\text{obs}}$ and $T_{\text{int}}$ (Figures 3(a) and 3(b)) for the different modules during the set of experiments at 1.5 bar.

Before the new injection of tracer, no influence of the pressure increase was visible on the transmission rates which were nearly the same before and after the TMP increase (Figure 3).

After the second tracer injection, no significant change in the observed removal $T_{\text{obs}}$ was observed by comparison to the first injection. This result is consistent with Arkhangelsky & Gitis (2008) who showed that the global virus removal was similar at 1 bar and at 2 bar for a T4 bacteriophage whose size is the closest to the tracer size used (64 nm, according to Soussan et al. 2011b).

On the contrary, for all the damaged modules, the intrinsic removals of the modules were better during the second injection than during the first injection (enhanced by a factor of 2 to 3) (Figures 2(b) and 3(b)). This phenomenon could be attributed to a tracer accumulation in the fibres and a possible fouling of the defects under the effect of the pressure increase. No fouling could however be detected by the permeability measurements because the holes had little impact on the global permeate flow rate (Table 1).

CONCLUSION

The innovative method implemented in this work permitted, in a very short time (about 10 min) diagnosis of a 50 μm hole in an ultrafiltration (UF) micromodule. Moreover, this method allowed characterization of the dynamics of virus removal for the different UF micromodules tested (with and without defect). It was notably evidenced that, whatever the applied TMP, removal performances were all the more enhanced than the defect size was small. It was also demonstrated that, whatever the defect size, the application during the filtration of a TMP step rise from 1 to 1.5 bar led to no significant change in the observed tracer removal but improved the intrinsic removal performances, probably due to a fouling of the defaults. This study confirms the dynamic behaviour of virus removal and the necessity to characterize and discuss virus removal in relation with the membrane and process history.

On the basis of trials performed at lab scale, this method appears as an interesting tool to detect defects and to study the dynamics of virus removal in relation with membrane properties, fouling and process history. Some further studies will focus on the improvement and adaptation of the method to larger scale modules and to check if it could be applied for the assessment of the inline virus removal in filtration plants for drinking water production.

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