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# Supercritical fluid treatment of organic membranes

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## ABSTRACT

Membrane processes are used for drinking water production and medical applications to remove and/or produce viruses using organic membranes. It appears that backwashing is not sufficient to remove all the viruses stopped by the membrane. The use of “one-shot” membranes or chlorinated solution filtration can be considered with an additional cost and accelerated aging of the membranes respectively. The inactivation of bacteria, spores and/or viruses has been demonstrated by the use of supercritical fluid. The use of a supercritical CO<sub>2</sub> treatment of organic membranes is studied with the aim to investigate the impact of this treatment on the membrane performances and on virus destruction. It is demonstrated that whatever the operating conditions, the membrane materials and the application modes, the permeability and the retention of the membrane are not impacted. TEM observation of the viruses after treatment shows no virus integrity.

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## 1. Introduction

Ultrafiltration is a process widely used in industrial applications and particularly for water treatment. Its performance in removing bacteria [1] and virus [2,3], and producing a stable and high quality permeate [4]. To replace conventional production processes, ultrafiltration (UF) is the most widely used process in drinking water production (Mierzwa et al., 2012). Hollow fibres and organic membranes are generally preferred because less expensive and with a higher compacity [5]. Moreover, even if they are more sensible to temperature and extreme pH conditions, mechanical properties are interesting because they combine robustness and flexibility and present a resistance to chemicals such as chlorin, used for chemical cleanings, in reasonable concentrations. Initially made in cellulose acetate since their introduction in the 90s, the most frequently used membranes nowadays are polyether sulfone (PES) and polyfluorure vinylidene (PVDF) [6]. Beside water treatment, ultrafiltration is increasingly used in medical applications [7]. However, membrane process presents an inconvenient: the membrane fouling which is the limiting factor for the ultrafiltration development [8]. Inactivating and/or eliminating virus and bacteria settled on membrane surface or inside membranes pore might be necessary in different conditions: for example, in biomedical applications or when the membrane process is stopped for a long time and microorganisms must be eliminated to prevent biofouling and membrane degradation over time for example and to obtain sterilizable and safe membranes in the fields of drinking water, food and pharmaceutical industry (Hicke et al., 2002).

There is indeed, in medicine, pharmaceutical and food industry a strong demand for safely sterilizable membranes. The use of oxidative chemicals is one of the solution to clean membranes but they have been shown to have negative impacts on membrane stability in terms of surface morphology and membrane performances [9]. UV radiations is known to be effective to inactivate microorganisms but [10] demonstrated that the structure and the performance of polymeric membranes were modified by this process even within few minutes (2 min) of exposure. To face those sterilization problems Hicke *et al.* [60] even investigated the preparation of a polymer membrane resistant to solvent and autoclave treatment. Supercritical fluids with intermediate properties between those of liquids and gases are present in a broad range of industrial application [11] such as extraction of components of interests from microalgae [12] or oil from argan seeds [13], medical applications such as the elaboration of lutein-loaded nanoliposomes [14] or the production of sustained drug delivery [15]. The possibility of inactivating microorganisms using supercritical fluids is known for decades with Dean Fraser who validated the use of CO<sub>2</sub> under pressure for the inactivation of *E.coli* [16]. Since, its efficiency for microorganisms inactivation was validated for bacteria [17] and even virus [18,19] and bacterial spore [20,21]. Among the different fluids studied, supercritical CO<sub>2</sub> (SCCO<sub>2</sub>) is a key interest considering that supercritical conditions are reached at mild pressure ( $P = 7.39$  MPa) and temperature ( $T = 31.1$  °C). Moreover, unlike organic solvents, SCCO<sub>2</sub> has several advantages: “green”, totally neutral, non-toxic, non-polluting and non-flammable. Therefore, there is an increasing interest for the

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sterilization of materials using supercritical fluids especially in medical applications where conventional treatments such as steam, ethylene oxide or gamma irradiation, may impact the integrity of the treated material [22–24]. For instance, Bennet *et al.* [25] investigated the sterilization of personal protective equipment (PPE) (surgical masks, cloth masks, N95 respirators) the SCCO<sub>2</sub> process used with additives led to a complete inactivation of coronavirus HCoV-NL63 and SARS-CoV-2 with no modification observed on PPE integrity (morphology and topographical structure). The use of additives may be necessary to reach complete sterilization or modify other parameters. For instance, Hemmer *et al.* [20] showed that with a temperature of 105 °C, two spores, *Geobacillus stearothermophilus* and *Bacillus atrophaeus* spores were completely inactivated with SCCO<sub>2</sub> at 300 bar in 25 min. With added H<sub>2</sub>O<sub>2</sub> (<100 ppm), 6 log populations of the two targeted spore types were inactivated in 1 h with a reduced temperature of 40 °C. [26] studied SCCO<sub>2</sub> treatment (100 bar, 35 °C) to eliminate biofouling on reverse osmosis membranes and obtained a reduction of *P. aeruginosa* biofilm cells by > 8 log within 30 min without a significant impact on RO membrane. Different factor can influence the sterilization efficiency using supercritical treatment such as temperature, pressure, depressurization rate, pressure cycling, temperature, presence of additives or treatment time, [27]. Modifying those parameters impact the performance of sterilization, the impact on material resistance but also the cost of the treatment.

The objective of this work is to study the membrane resistance to supercritical treatment under different treatment conditions and the feasibility of using SCCO<sub>2</sub> to inactivate virus on ultrafiltration membranes. Two membrane materials were studied: polyethersulfone (PES) and PVDF. After verifying the resistance of the membranes to SCCO<sub>2</sub> treatment, the inactivation of Tulane virus, a surrogate of norovirus was studied. The choice of viruses was explained by two reasons: they appear to be more resistant than bacteria [27] and their size is the same magnitude as the membrane pore size around 20 nm. The objective was two-fold: evaluate (i) the resistance of the membranes to supercritical treatment under different treatment conditions; hydraulic and retention performances were controlled and (ii) the efficiency of the process to inactivate the targeted virus using qPCR analyses and MEB observations. Treatment conditions (pressure, temperature, treatment time, continuous/semi-continuous) were chosen regarding the resistance of membrane materials. The feasibility of organic membranes treatment with supercritical CO<sub>2</sub> is, for the first time, evaluated on the conservation of the membrane properties and on the inactivation of the viruses retained by the membranes.

## 2. Materials and methods

### 2.1. Virus

The virus preparations and analyses are realized in the Laboratoire de Microbiologie, LSEM-SG2M (Ifremer, Nantes, France) [28]. In some studies, it is shown that Tulane virus may be a good surrogate for studying norovirus and the prolonged persistence of Norwalk virus in oyster tissues is validated.

#### 2.1.1. Viruses, cell culture preparation and analyses

Tulane virus (TV) strain M033 as previously described [29,30].

This virus preparation and analysis is well described [3] and realized in three parts: (i) Nucleic-acid (NA) extraction, (ii) primers, probes and rRT-PCR and (iii) rRT-PCR controls and quantification.

#### 2.1.2. Transmission Electron Microscopy

Protocol for the negative staining on Tulane virus is realized in three steps: (i) an ultra-centrifugation of the solutions is realized at 60 000 RPM during 5 min. (ii) 3 µL of the centrifugated solution are deposited for 3 min on 200 mesh nickel grids coated with a Formvar film. Before this deposit, a 15 mA glow discharge is applied on this grid in a sealed

chamber composed of argon during 40 s. Uranyl acetate 1% in distilled water is added during 1 min. The excess dye is absorbed with ashless filter paper. (iii) the observation is carried with a Transmission Electron Microscopy (TEM FEI G2 200kv).

### 2.2. Ultrafiltration membranes, modules and retention

#### 2.2.1. Membranes and modules

2 membranes, used industrially for the production of drinking water, are used and the characteristics are given in Table 1. We chose asymmetric and anisotropic membrane, because in the case of SCCO<sub>2</sub> treatment of composite membrane with different layers, a risk of delamination during foaming was observed [31]. The filtration of these membranes takes place in the internal-external direction and in external-internal direction respectively for the membrane A and B. Membrane modules were made of membrane A or B into a PTFE Tube. Epoxy plugs are realized to allow in/out frontal filtration and out/in frontal filtration respectively. After the module potting, membranes were rinsed (300 L m<sup>-2</sup>) to remove glycerin as mentioned [32]. Two pilot plants (Fig. 1) were used in agreement with the filtration mode, dead-end for the permeability measurement, filtration of virus whatever the membrane geometry or for dextran retention with single channel membrane and cross flow for dextran retention test for multichannel membrane filtration. The membrane module (7) is fed from a tank (4) pressurized with air (1). The pressure is measured by manometers (2-6-9) and is regulated by pressure valves (3-5-8-10). The feed rate (A) is equal to the permeate rate (B). For crossflow filtration, the configuration, shown in Figs. 1–2., allows operation with controlled velocity and transmembrane pressure. The membrane module (5) is fed by a centrifugal pump (2) from a tank (1) containing the solution. The pressure is measured by manometer (4-7-9) and is regulated by pressure valves (3-8-10). The feed flow (A) measured by a flow meter (6) is divided into a permeate flow (B) and a retentate flow (C).

#### 2.2.2. Virus filtration

For each experiment, a new module/membrane was used and the viral concentration in the feed was from 1.0 10<sup>5</sup> to 3.47 10<sup>7</sup> RNA copies mL<sup>-1</sup> due to the volumic concentration factor. All filtration experiments were performed in vertical dead-end filtration mode using the lab-scale setup presented in Fig. 1 to be close to production mode. Pure pressurized air was connected to the tank containing virus suspensions which was itself connected to the module. Dead-end filtration was carried out at a constant TMP (0.3 bar). The permeate mass was recorded and all experiences were carried out at room temperature (20 °C ± 2 °C). At the end of the filtration, the membrane module was disconnected and the retentate contained in fiber channels was recovered by backwash with pure water. From an initial volume of feed solution of 480 mL, 80 mL are used to validate the initial concentration (80 mL is the minimal value for qPCR). The permeate is collected over time. After filtration, a backwash of 80 mL is recovered and sampled. This protocol was similar for the two filtration modes used (i.e. membranes used).

**Table 1**  
Membrane characteristics.

	Membrane A	Membrane B
Geometry	Polymeric multichannel hollow fibers (7 channels)	Single channel
Material	PES	PVDF
Internal – external diameter (mm)	0.9–4	0.38–0.72
Membrane permeability (L h <sup>-1</sup> m <sup>-2</sup> bar <sup>-1</sup> )	700 ± 100	500 ± 50
Pore size (nm)	20	15
Number of membranes per module	1	30
Dead end filtration mode	Int-Ext	Ext-Int

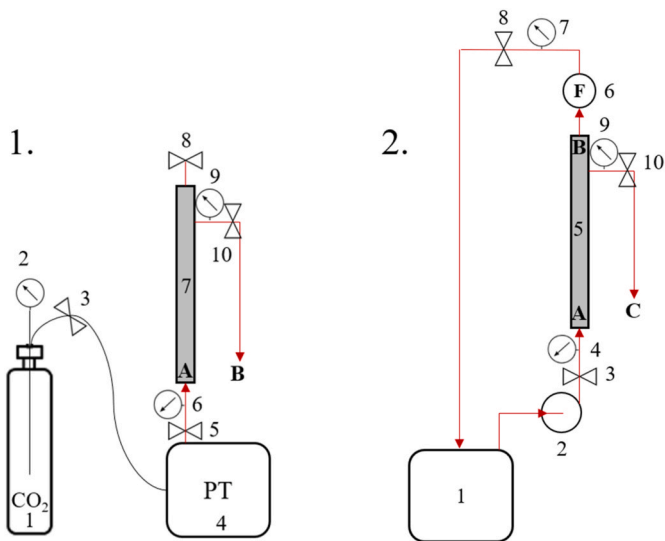


Fig. 1. Filtration pilots used.

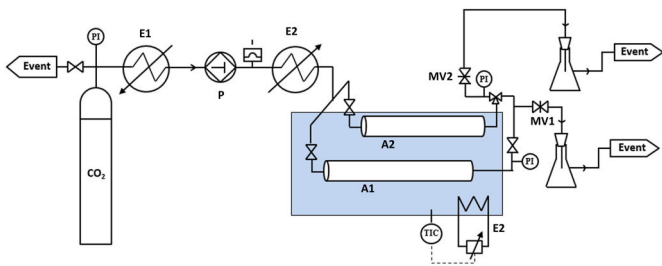


Fig. 2. Process diagram of the experimental setup: (E1) cooler, (E2) hot circulator bath, (P) CO<sub>2</sub> high pressure liquid pump, (A1 and A2) high pressure tubes, (MV1 and MV2) micrometric valve.

### 2.2.3. Permeability

Before each experiment the initial membrane permeability is evaluated with Evian water by drawing the variation of the permeate flux at 20 °C as the function of the TMP. After each SCCO<sub>2</sub> treatment, a measurement of permeability was realized and compared to the initial value.

### 2.2.4. Retention evaluation by dextran filtration

The membrane module was rinsed during 15 min with a transmembrane pressure of approximately 1 bar and the initial permeability was measured. The temperature of the experiment was 20 °C. In the 1 L beaker, 6 g of T500 dextran ( $C = 2.0 \text{ g L}^{-1}$ ) and 0.3 g of NaNO<sub>3</sub> ( $C = 0.1 \text{ g L}^{-1}$ ) were weighted and approximately 500 mL of ultrafiltered water to dissolve the solutes was added. After a shake with a magnetic stirrer during 15 min, the solution was transferred to the 5 L beaker and make up to 3 L with ultrafiltered water. A feed sample (about 20 mL) was collected: it is to measure the value of the feed solution. The feed tank was feed with about 2.5 L of the dextran solution (20 °C). A feed screw pump ensures the fluid circulation and controls the fluid velocity into the module (Reynolds number = 1,500). The retentate and the permeate of the membrane module were recycled in the feed tank. The transmembrane pressure was gradually increased by closing the valve (0.3, 0.6 and 1 bar). The circulation flow rate was measured using a float flowmeter and permeate mass by using an electronic balance ( $\Delta m = \pm 0.01 \text{ g}$ ). After 15 min of filtration at the considered PTM, the inlet and outlet pressures were recorded and a permeate was sampled (about 20 mL) for the retention measurement before the gradually increase of the TMP.

A high precision densimeter (DMA 5000 M) which a precision of  $0.000005 \text{ g cm}^{-3}$  was used to determine the dextran concentration by

density and volumic weight measurements (References: air density =  $0.001192 \text{ g cm}^{-3}$  at 20 °C and distilled water density =  $0.998192 \text{ g cm}^{-3}$  at 20 °C). Before each measurement, the device reliability was verified with distilled water. A refractometer (Anton Paar - Abbemat 500) was also used. So, the dextran retention was calculated using refractometer index, density and volumic weight. The retention was the same using by these 3 parameters.

### 2.3. SCCO<sub>2</sub> treatment

The membranes treatment by SCCO<sub>2</sub> is carried out in autoclaves charged with CO<sub>2</sub> in batch mode. The experimental set-up is shown in Fig. 2. Initially the membranes were placed in high pressure tubes (internal diameter: 0.8 cm, length: 30 cm - A1 and A2). Once the tubes were reconnected to the set-up, they were charged with CO<sub>2</sub> until the working pressure was reached (100 or 150 bar). The CO<sub>2</sub> was stored in liquid form in high pressure tanks. It is then cooled in a cold bath (Selecta Frigiterm - E1) to  $-5.5 \text{ °C}$  before reaching the high-pressure liquid pump (Jasco PU-4386 - P). At the pump outlet, the CO<sub>2</sub> was preheated to working temperature (35 °C) thanks to a hot bath operating with an immersed heating resistance (Fisherbrand Polystat - E2) before being sent to the tubes. The CO<sub>2</sub> injection was stopped when the pressure in the tubes containing the membranes reaches the working pressure. Once the working pressure was achieved, the tubes were isolated from the rest of the process by valves and the membranes remain in contact with the CO<sub>2</sub> during the different treatment times (20 min and 2 h). The autoclaves were immersed in the hot bath (E2) in order to maintain a temperature of 35 °C during all the experiment. When the time was achieved, the whole system was depressurized with two micrometric valves (MV1 and MV2) by keeping a depressurization rate of  $5 \text{ bar min}^{-1}$  during the complete depressurization process. The microvalves arrangement allows to depressurize at several time the two autoclaves. The membranes were finally recovered for characterization when the system return to atmospheric pressure.

## 3. Results

### 3.1. Effect of supercritical fluid on membrane performances

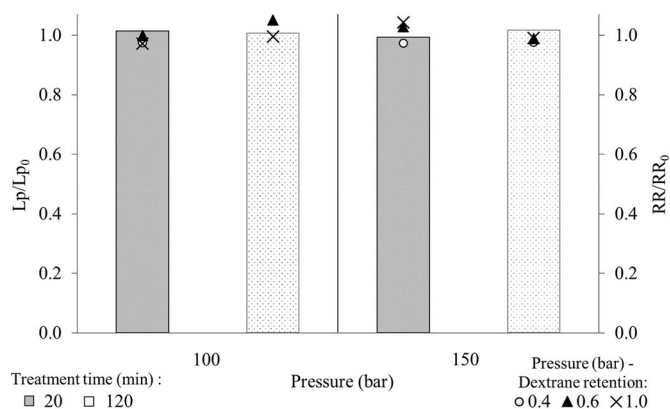
First, the performance of the membrane in terms of permeability and retention will be evaluated before and after supercritical treatment. For this purpose, the ratios  $L_p/L_{p0}$  and  $RR/RR_0$  representing respectively the ratio between the permeability and the initial permeability and the ratio between the retention and the initial retention will be presented according to the operating conditions.

#### 3.1.1. Impact of treatment conditions (pressure and contact time) and membrane material

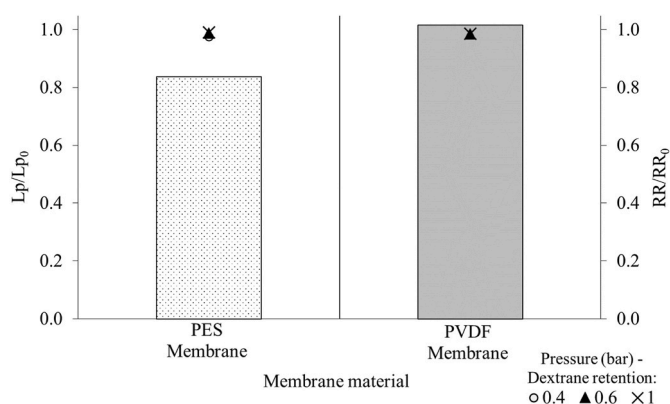
Since the membranes are organic, the temperature for the experiments was set at 35 °C. A temperature of 35 °C allows to work with CO<sub>2</sub> in supercritical form while respecting the operating temperature range of the membranes given by the manufacturer. The conditions of pressure and contact time were chosen according to the literature [27]. Fig. 3 shows that whatever the conditions of pressure and contact time, the retention and permeability values remain constant. It should be noted that the retention was evaluated by density measurements and refractometry. These conclusions remain valid regardless of the membrane material for the highest-pressure condition as shown in Fig. 4.

#### 3.1.2. Impact of operating mode

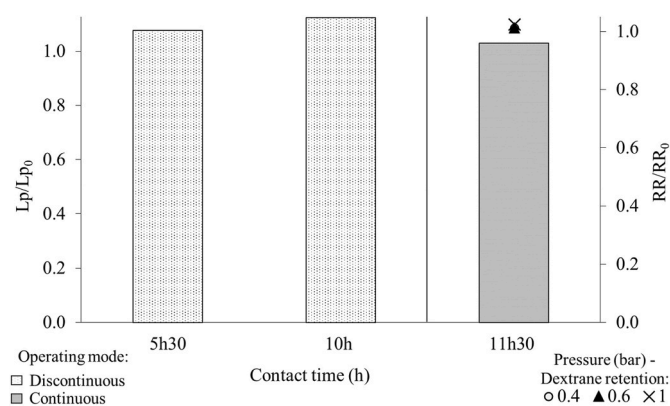
SCCO<sub>2</sub> for membrane treatment could be used sequentially after filtration or after chemical regeneration or backwash. Also, the same experiments were conducted on several cycles in batch (the first cycle consisting of 1h30 + 2 h + 2 h treatment time and the second cycle composed of 3 h + 4 h + 4 h treatment time) and continuous (11h30 treatment time) mode. Fig. 5 shows that whatever the mode and the



**Fig. 3.** Variation of permeability and dextran retention after SCCO<sub>2</sub> treatment for different pressures and contact times [PES membrane].



**Fig. 4.** Variation of permeability and dextran retention after SCCO<sub>2</sub> treatment for different membrane material [Pressure = 150 bar, T = 35 °C, contact time = 2 h].



**Fig. 5.** Variation of permeability and dextran retention after SCCO<sub>2</sub> treatment for different operating mode [Pressure = 150 bar, T = 35 °C].

contact time of the SCCO<sub>2</sub> treatment, the permeability and retention of the membrane were not impacted.

Regarding the different applications combining membrane processes and SCCO<sub>2</sub>, such as membrane reactor/contactors, supercritical assisted ultrafiltration, solvent recovery or extraction, inorganic membranes are preferred because of their higher stability. Organic membranes also have their place in supercritical conditions but their mechanical and chemical resistance must be verified under the conditions of pressure and temperature applied [33–36]. As expected, the membrane compaction does

not occur, no modification of the permeability, due to the fact that only a contact between membrane and SCCO<sub>2</sub> occur and not a filtration.

Then, evaluating the retention and permeability of ultrafiltration membrane after treatment in this study is essential to guarantee that SCCO<sub>2</sub> doesn't impact membrane integrity with the applied operating conditions. Supercritical CO<sub>2</sub> properties (density close to liquid density and diffusivity close to gas diffusivity) allows a fast solubilization of SCCO<sub>2</sub> in polymers [58]. These strong solubilities may cause plasticization and swelling of materials, with a modification of the mechanical and physical properties with a lower glass transition temperature and melting temperature [37,38]. Poly(vinylidene fluoride) (PVDF) have a high melting temperature ( $T_m$ ) at 160–180 °C. According to Shieh *et al.* [39], although  $T_m$  of PVDF is near 160 °C, melting is observed at 140 °C under pressure conditions of CO<sub>2</sub> of 283 and 473 atm. The CO<sub>2</sub>-assisted melting of PVDF recrystallizes during depressurization of CO<sub>2</sub>, leading to an increased thickness of crystal layers but an insignificant change in thickness of amorphous layers. These results were confirmed by Shenoy *et al.* [40] that observed a minimum  $T_m$  of 135 °C at 483 bar. The glass transition temperature of PES is 503 K, and this polymer presents a good chemical and thermal stability. Modification of PES was observed with membranes prepared for gas separation with polyamide copolymer layer or a polyvinyl alcohol (PVA) layer on top of the PES support membrane exposed to SCCO<sub>2</sub> at 15 MPa and 313 K for 6–8 h. A flux increment of about 30–50% polymeric structure is changing, traducing swelling and plasticization effects of layers [41].

The condition applied in the present study (pressure maximum = 150 bar, temperature of 35 °C) are significantly softer and confirm these results. However, according to Masmoudi *et al.* [42] the presence of co-solvent such as water and the depressurization rate are parameters that will influence the resistance polymer to supercritical treatment. The depressurization rate applied (5 bar min<sup>-1</sup>) is considered low by the same authors and the presence of water within the membrane didn't lead to membrane degradations according to the analyses realized. PES and PVDF membranes resist to SCCO<sub>2</sub> treatment with the chosen conditions applied of pressure, temperature, presence of water, contact time, pressurization/depressurization cycles and depressurization rate. The other materials that compose PES membrane module are polyvinyl chloride and/or polyolefine. These specific materials composing the membrane module have not been exposed to the SCCO<sub>2</sub> conditions, so it is difficult to conclude on their stability but some informations can be found in literature. Polyolefin fibres show a good stability in supercritical CO<sub>2</sub> at temperatures of up to 120 °C and pressures of 280 bar [43].

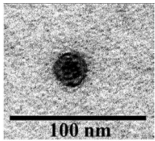
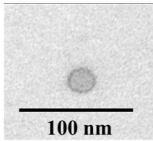
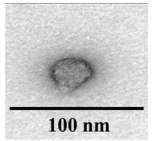
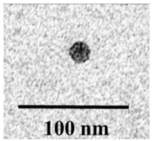
PVC is a thermosensitive polymer and may face modification when treated with SCCO<sub>2</sub> in the conditions of pressure, temperature and contact time tested in the study [44] but also depend of the depressurization rates. Therefore, the stability of the specific components of the module will have to be effectively conducted.

### 3.2. Inactivation of virus

Virus filtration was conducted on Tulane viruses, surrogated for human norovirus, for (i) safety of use and (ii) this virus is well retained by PES membrane for high concentration [3]. Microscopic observations (TEM) (Table 2) of the solutions obtained after filtration and backwash show integrated viruses presenting a spherical capsid of 30 nm in diameter in agreement with literature data. In order to study the virus inactivation on membranes by SCCO<sub>2</sub> treatment, it would have been preferable to analyse by TEM the membrane surface. Microscopic observations of organic membranes for sizes close to nm (viruses and pore size) are difficult [45–47], and require special preparations before observations (metal deposition under vacuum) which could have impacted the integrity of the viruses. To avoid these effects, backwash water was studied even if we cannot conclude that no virus will remain on the membrane after SCCO<sub>2</sub> treatment. After passage through the SCCO<sub>2</sub> treatment, it appears that these integrated viruses are no longer present and show either an empty capsid, destroyed or very small sizes of the

**Table 2**TEM observation of the backwash solution after SCCO<sub>2</sub> treatment for different pressures [PES membrane, T = 35 °C, Contact time = 2 h].

Operating conditions - Pressure (bar)	Active virus (%)	Empty virus (%)	Destroyed virus (%)	Virus with a size <20 nm (%)
100	0	46.7	33.3	20.0
150	0	30.8	50.0	19.2

order of 10–15 nm indicating the destruction of the viruses. TCID 50 (Median Tissue Culture Infectious Dose) analyses were realized to verify the viral titer. After cultivation of cells on well plate and incubation, the percentage of infected wells was determined for several dilutions, to obtain the TCID50. The monitoring of virus growth on culture media for 7 days for a SCCO<sub>2</sub> treatment (2 h, 150 bar, 35 °C, PES membrane) show a non-infectivity of the virus, but these preliminary results must be confirmed with replicate.

The proposed mechanisms of microbial inactivation by supercritical fluids are, first, the acidification of the medium during SCCO<sub>2</sub> treatment that impact the resistance of microorganisms to inactivation and increase cell permeability. Following this membrane rupture leading to the penetration of CO<sub>2</sub> within microbial cell, a reaction with cellular materials results in a reduction of enzymatic activities, cell tear and damage [27,48,49]. CO<sub>2</sub> affinity and permeation within the cell membrane has been re-reported in different types of cells [50]. In the case of virus, SCCO<sub>2</sub> interactions with envelop seem to be the principal source of inactivation. Moreover [51], put in light further evidence that pH changes caused by CO<sub>2</sub> solubility may involve the enhancement of viral inhibition. Indeed, pressurized CO<sub>2</sub> was shown efficient to inactivate bacteriophages with higher performance than NO<sub>2</sub> in similar conditions, the main difference was the pH reached within water during supercritical treatment. Furthermore, the inactivation efficiency was also compared with HCl and, even though identical pH values were obtained in the two conditions, pressurized CO<sub>2</sub> led to a higher reduction of virus. This is explained by the authors because normal acids are not able to penetrate the protein coat of phage. Inactivation of microorganisms by SCCO<sub>2</sub> is linked to conditions of treatment (pressure, temperature, contact time and cycles of pressurization/depressurization), the kind of microorganism and the presence of co-solvent. Indeed, the use of SCCO<sub>2</sub> may not be efficient to reach total sterilization especially regarding the inactivation of bacterial spores and virus which are more resistant to physical treatments. Fages *et al.* [52] validated the inactivation using SCCO<sub>2</sub> of four viruses, enveloped and non-enveloped (HIV-1, Sindbis, Polio Sabin type I and Pseudorabies) at a higher pressure, 250 bar, 50 °C and a contact time of 10 min. Inactivation of virus was obtained without additive under operating conditions similar to those applied for this study. For instance, at temperatures of 40–50 °C, a pressure of 160 bar for 45 min, SCCO<sub>2</sub> lead to a virus inactivation in a heat-sensitive biomaterial [27] but for enveloped viruses which are less resistant than non-enveloped viruses, like the targeted Tulane virus in this work.

Then, the use of SCCO<sub>2</sub> combined with a sterilant or a co-solvent, such as peracetic acid or hydrogen peroxide, can be necessary [19, 53]. Among the studied co-solvent, water was proved to be a key factor for inactivation of bacteria and fungo spores [50,54,55]. This indicates that water is key to this treatment, as it serves as the medium in which the microbial cells are inactivated [56,57]. Also, in the present study, as our membranes are not dried between the virus filtration step and the SCCO<sub>2</sub> treatment, water is still present in pores and may act as a co-solvent enhancing virus inactivation. The SCCO<sub>2</sub> treatment resulted in an inactivation of non-enveloped viruses with relatively low operating conditions in terms of contact time, temperature, and pressure. As this kind of virus is known to be more resistant than enveloped viruses

and bacteria, an efficient inactivation of these microorganisms too on ultrafiltration membrane could be expected with these treatment conditions: temperature of 35 °C, pressure 100 or 150 bar and contact time of 20 min or 120 min, depressurization rate of 5 bar min<sup>-1</sup> and absence of drying step of the membranes before treatment.

For commercial uses, as in the case of water treatment, few viruses remain on the membrane surface after a backwash [2], pharmaceutical/medical applications will be more targeted. In this case, a module with a large specific area (m<sup>-1</sup>) is used with a low volume and are suitable for autoclave treatment as implemented in this study. To be noted that the inactivation efficiency was evaluated only with a solution containing only viruses. In the case of more complex solution (macromolecules, colloidal material and/or particles), difficult SCCO<sub>2</sub> access to microorganisms might be observed [27,59].

#### 4. Conclusion

The SCCO<sub>2</sub> treatment proved to be a possible robust alternative to the organic membrane for virus inactivation. The hydraulic and retention performances are maintained after a SCCO<sub>2</sub> treatment whatever the operating conditions of pressure (100–150 bar) and contact time (20, 120, 660 min), for a temperature of 35 °C, whatever the membrane material (PES, PVDF) studied and whatever the operating mode (continuous, semi-continuous). It is the first time that these results of impact of SCCO<sub>2</sub> on hydraulic performances are presented for organic membranes. Regarding the inactivation of viruses, SEM analyses showed the performance of SCCO<sub>2</sub> treatment in our conditions toward Tulane virus with an absence of visible active virus. Acidification of the medium during SCCO<sub>2</sub> treatment may be the mechanism of inactivation of Tulane virus and the presence of water within membranes may enhance the treatment performance. It must be noted that the observation of virus inactivation was only realized on virus recovered from backwash and not the totality of virus because a part of them might be still present on membrane, even the SCCO<sub>2</sub> is present everywhere around the membrane. This present work aimed to demonstrate the feasibility of SCCO<sub>2</sub> treatment of organic membranes. The next step would be the study of complex matrix containing virus, the validation of the module stability considering the presence of other components and more detailed analysis of the viruses in the membrane to tend towards a sterilization of the membrane. Finally, deeper analyses could be performed to assess the total integrity (ATR-FTIR spectra, FE-SEM surface morphological imaging) and surface properties of the membrane (hydrophilicity/hydrophobicity, surface roughness, and surface charge).

#### Author contribution

Mahieu MARTINO: Investigation, Experimentation, Writing the original draft. Hugo TALIGROT: Experimentation, treatment of data, Experimentation, writing and review. Clemence CORDIER: Co supervision, Experimentation, Writing the original draft, and treatment of the results. Philippe MOULIN: Co supervision, Experimentation, Writing the original draft, Treatment of the results, Resources.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## References

- [1] S.S. Madaeni, The application of membrane technology for water disinfection, *Water Res.* 33 (1999) 301–308, [https://doi.org/10.1016/S0043-1354\(98\)00212-7](https://doi.org/10.1016/S0043-1354(98)00212-7).
- [2] N. Jacquet, S. Wurtzer, G. Darracq, Y. Wyrat, L. Moulin, P. Moulin, Effect of concentration on virus removal for ultrafiltration membrane in drinking water production, *J. Membr. Sci.* (2021), <https://doi.org/10.1016/j.memsci.2021.119417>, 119417.
- [3] H. Taligrot, M. Monnot, J. Ollivier, C. Cordier, N. Jacquet, E. Vallade, P. Garry, C. Stravakakis, F.S. Le Guyader, P. Moulin, Retention of the Tulane virus, a norovirus surrogate, by ultrafiltration in seawater and production systems, *Aquaculture* 553 (2022), 738096, <https://doi.org/10.1016/j.aquaculture.2022.738096>.
- [4] C.M. Chew, M.K. Aroua, M.A. Hussain, W.M.Z.W. Ismail, Evaluation of ultrafiltration and conventional water treatment systems for sustainable development: an industrial scale case study, *J. Clean. Prod.* 112 (2016) 3152–3163, <https://doi.org/10.1016/j.jclepro.2015.10.037>.
- [5] P.T. Cardew, *Membrane Processes: A Technology Guide*, Royal Society of Chemistry, 2007.
- [6] G. Pearce, Introduction to membranes: membrane selection, *Filtr. Sep.* 44 (2007) 35–37, [https://doi.org/10.1016/S0015-1882\(07\)70083-6](https://doi.org/10.1016/S0015-1882(07)70083-6).
- [7] S.A. Aani, T.N. Mustafa, N. Hilal, Ultrafiltration membranes for wastewater and water process engineering: a comprehensive statistical review over the past decade, *J. Water Proc. Eng.* 35 (2020), 101241, <https://doi.org/10.1016/j.jwpe.2020.101241>.
- [8] W. Gao, H. Liang, J. Ma, M. Han, Z. Chen, Z. Han, G. Li, Membrane fouling control in ultrafiltration technology for drinking water production: a review, *Desalination* 272 (2011) 1–8, <https://doi.org/10.1016/j.desal.2011.01.051>.
- [9] M.T. Tsehaye, S. Velizarov, B. Van der Bruggen, Stability of polyethersulfone membranes to oxidative agents: a review, *Polym. Degrad. Stabil.* 157 (2018) 15–33, <https://doi.org/10.1016/j.polydegradstab.2018.09.004>.
- [10] N.N. Rupiasih, H. Suyanto, M. Sumadiyasa, N. Wendri, Study of Effects of Low Doses UV Radiation on Microporous Polysulfone Membranes in Sterilization Process, 2013, <https://doi.org/10.4236/ojpm.2013.31003>, 2013.
- [11] A.A. Clifford, J.R. Williams, Introduction to supercritical fluids and their applications, in: *Supercritical Fluid Methods and Protocols*, Springer, 2000, pp. 1–16.
- [12] A. Mouahid, C. Crampon, S.-A.A. Toudji, E. Badens, Supercritical CO<sub>2</sub> extraction of neutral lipids from microalgae: experiments and modelling, *J. Supercrit. Fluids* 77 (2013) 7–16, <https://doi.org/10.1016/j.supflu.2013.01.024>.
- [13] A. Mouahid, I. Bombarda, M. Claeys-Bruno, S. Amat, E. Myotte, J.-P. Nisteron, C. Crampon, E. Badens, Supercritical CO<sub>2</sub> extraction of Moroccan argan (*Argania spinosa* L.) oil: extraction kinetics and solubility determination, *J. CO<sub>2</sub> Util.* 46 (2021), 101458, <https://doi.org/10.1016/j.jcou.2021.101458>.
- [14] M. Martino, A. Mouahid, P. Trucillo, E. Badens, Elaboration of lutein-loaded nanoliposomes using supercritical CO<sub>2</sub>, *Eur. J. Lipid Sci. Technol.* 123 (2021), 2000358, <https://doi.org/10.1002/ejlt.202000358>.
- [15] K. Ongkasin, Y. Masmoudi, C.M. Wertheimer, A. Hillenmayer, K.H. Eibl-Lindner, E. Badens, Supercritical fluid technology for the development of innovative ophthalmic medical devices: drug loaded intraocular lenses to mitigate posterior capsule opacification, *Eur. J. Pharm. Biopharm.* 149 (2020) 248–256, <https://doi.org/10.1016/j.ejpb.2020.02.011>.
- [16] D. Fraser, Bursting bacteria by release of gas pressure, *Nature* 167 (1951) 33–34, <https://doi.org/10.1038/167033b0>.
- [17] Y.Y. Chen, F. Temelli, M.G. Gänzle, Mechanisms of inactivation of dry *Escherichia coli* by high-pressure carbon dioxide, *Appl. Environ. Microbiol.* 83 (2017), <https://doi.org/10.1128/AEM.00062-17>.
- [18] T.P. Castor, A.D. Lander, M.D. Cosman, P.R. D'entremont, M.R. Pelletier, *Viral Inactivation Method Using Near Critical, Supercritical or Critical Fluids*, 1999.
- [19] Q.-Q. Qiu, P. Leamy, J. Brittingham, J. Pomerleau, N. Kabaria, J. Connor, Inactivation of bacterial spores and viruses in biological material using supercritical carbon dioxide with sterilant, *J. Biomed. Mater. Res. B Appl. Biomater.* 91B (2009) 572–578, <https://doi.org/10.1002/jbm.b.31431>.
- [20] J.D. Hemmer, M.J. Drews, M. LaBerge, M.A. Matthews, Sterilization of bacterial spores by using supercritical carbon dioxide and hydrogen peroxide, *J. Biomed. Mater. Res. B Appl. Biomater.* 80B (2007) 511–518, <https://doi.org/10.1002/jbm.b.30625>.
- [21] J. Zhang, S. Burrows, C. Gleason, M.A. Matthews, M.J. Drews, M. LaBerge, Y.H. An, Sterilizing *Bacillus pumilus* spores using supercritical carbon dioxide, *J. Microbiol. Methods* 66 (2006) 479–485, <https://doi.org/10.1016/j.mimet.2006.01.012>.
- [22] B. Buszewski, O. Wrona, R.P. Mayya, A.M. Zakharenko, T.K. Kalenik, K. S. Golokhvast, W. Piekoszewski, K. Rafińska, The potential application of supercritical CO<sub>2</sub> in microbial inactivation of food raw materials and products, *Crit. Rev. Food Sci. Nutr.* (2021) 1–14, <https://doi.org/10.1080/10408398.2021.1902939>.
- [23] N. Ribeiro, G.C. Soares, V. Santos-Rosales, A. Concheiro, C. Alvarez-Lorenzo, C. A. Garcia-González, A.L. Oliveira, A new era for sterilization based on supercritical CO<sub>2</sub> technology, *J. Biomed. Mater. Res. B Appl. Biomater.* 108 (2020) 399–428, <https://doi.org/10.1002/jbm.b.34398>.
- [24] A. White, D. Burns, T.W. Christensen, Effective terminal sterilization using supercritical carbon dioxide, *J. Biotechnol.* 123 (2006) 504–515, <https://doi.org/10.1016/j.jbiotec.2005.12.033>.
- [25] D. Bennet, A.F. Harris, J. Lacombe, C. Brooks, N. Bionda, A.D. Strickland, T. Eisenhut, F. Zenhausern, Evaluation of supercritical CO<sub>2</sub> sterilization efficacy for sanitizing personal protective equipment from the coronavirus SARS-CoV-2, *Sci. Total Environ.* 780 (2021), 146519, <https://doi.org/10.1016/j.scitotenv.2021.146519>.
- [26] S. Mun, Y. Baek, C. Kim, Y.-W. Lee, J. Yoon, Feasibility of supercritical CO<sub>2</sub> treatment for controlling biofouling in the reverse osmosis process, *Biofouling* 28 (2012) 627–633, <https://doi.org/10.1080/08927014.2012.700051>.
- [27] M. Perrut, Sterilization and virus inactivation by supercritical fluids (a review), *J. Supercrit. Fluids*, Special Edition on the Occasion of Gerd Brunner's 70th Birthday 66 (2012) 359–371, <https://doi.org/10.1016/j.supflu.2011.07.007>.
- [28] N. Drouaz, J. Schaeffer, T. Farkas, J.L. Pendu, F.S.L. Guyader, Tulane virus as a potential surrogate to mimic norovirus behavior in oysters, *Appl. Environ. Microbiol.* 81 (2015) 5249–5256, <https://doi.org/10.1128/AEM.01067-15>.
- [29] T. Farkas, K. Sestak, C. Wei, X. Jiang, Characterization of a rhesus monkey calicivirus representing a new genus of caliciviridae, *J. Virol.* 82 (2008) 5408–5416, <https://doi.org/10.1128/JVI.00070-08>.
- [30] L.R. Martin, G.M. Duke, J.E. Osorio, D.J. Hall, A.C. Palmenberg, Mutational analysis of the mengovirus poly(C) tract and surrounding heteropolymeric sequences, *J. Virol.* 70 (1996) 2027–2031.
- [31] J.L. Sumey, J.A. Sarver, E. Kiran, Foaming of polystyrene and poly(methyl methacrylate) multilayered thin films with supercritical carbon dioxide, *J. Supercrit. Fluids* 145 (2019) 243–252, <https://doi.org/10.1016/j.supflu.2018.12.001>.
- [32] S. Arenillas, M. Drouin, E. Monnin, P. Moulin, Glycerin removal from ultrafiltration flat sheet membranes by filtration and soaking, *J. Membr. Sci. Res.* 3 (2017) 102–108, <https://doi.org/10.22079/jmsr.2016.23080>.
- [33] M. Koh, B. Fournel, S. Sarrade, L. Schrive, I. Stoychev, P. Lacroix-Desmazes, T. Ribaut, K. Park, Continuous supercritical CO<sub>2</sub> process using nanofiltration by inorganic membrane, *Ind. Eng. Chem. Res.* 48 (2009) 5406–5414, <https://doi.org/10.1021/ie800452g>.
- [34] S. Sarrade, C. Guizard, G.M. Rios, New applications of supercritical fluids and supercritical fluids processes in separation, *Separ. Purif. Technol.* 32 (2003) 57–63, [https://doi.org/10.1016/S1383-5866\(03\)00054-6](https://doi.org/10.1016/S1383-5866(03)00054-6).
- [35] S. Sarrade, C. Guizard, G.M. Rios, Membrane technology and supercritical fluids: chemical engineering for coupled processes, *Desalination* 144 (2002) 137–142, [https://doi.org/10.1016/S0011-9164\(02\)00302-8](https://doi.org/10.1016/S0011-9164(02)00302-8).
- [36] S. Sarrade, G.M. Rios, M. Carlés, Nanofiltration membrane behavior in a supercritical medium, *J. Membr. Sci.* 114 (1996) 81–91, [https://doi.org/10.1016/0376-7388\(95\)00308-8](https://doi.org/10.1016/0376-7388(95)00308-8).
- [37] I. Kikic, Polymer-supercritical fluid interactions, *J. Supercrit. Fluids*, 20th Year Anniversary Issue of the Journal of Supercritical Fluids 47 (2009) 458–465, <https://doi.org/10.1016/j.supflu.2008.10.016>.
- [38] M. Sauceau, S. Puissant, D. Richon, J. Fages, Développement de procédés d'extrusion assistés par un fluide supercritique, in: *D. DUPUIS sous la dir de (Ed.), 39ème Colloque Annuel Du Groupe Français de Rhéologie, GROUPE FRANCAIS DE RHEOLOGIE*, Mulhouse, France, 2004, pp. 248–251.
- [39] Y.-T. Shieh, T.-T. Hsiao, S.-K. Chang, CO<sub>2</sub> pressure effects on melting, crystallization, and morphology of poly(vinylidene fluoride), *Polymer* 47 (2006) 5929–5937, <https://doi.org/10.1016/j.polymer.2006.06.022>.
- [40] S.L. Shenoy, T. Fujiwara, K.J. Wynne, Quantifying plasticization and melting behavior of poly(vinylidene fluoride) in supercritical CO<sub>2</sub> utilizing a linear variable differential transformer, *Macromolecules* 36 (2003) 3380–3385, <https://doi.org/10.1021/ma025929d>.
- [41] V.E. Patil, L.J.P. van den Broeke, F.F. Vercauteren, J.T.F. Keurentjes, Permeation of supercritical carbon dioxide through polymeric hollow fiber membranes, *J. Membr. Sci.* 271 (2006) 77–85, <https://doi.org/10.1016/j.memsci.2005.06.059>.
- [42] Y. Masmoudi, L. Ben Azzouk, O. Forzano, J.-M. Andre, E. Badens, Supercritical impregnation of intraocular lenses, in: *J. Supercrit. Fluids, Selected Papers from the 9th Conference on Supercritical Fluids and Their Applications*, September 05-08, 2010 vol. 60, Sorrento (Italy), 2011, pp. 98–105, <https://doi.org/10.1016/j.supflu.2011.08.014>.
- [43] E. Bach, E. Cleve, E. Schollmeyer, The dyeing of polyolefin fibers in supercritical carbon dioxide. Part I: thermo-mechanical properties of polyolefin fibers after

- treatment in CO<sub>2</sub> under dyeing conditions, *J. Text. Inst.* 89 (1998) 647–656, <https://doi.org/10.1080/00405000.1998.11090903>.
- [44] S.P. Sawan, Y.-T. Shieh, J.-H. Su, Evaluation of The-Interactions between Supercritical Carbon Dioxide and Polymeric Materials 144, 1994.
- [45] R. Tamime, Y. Wyart, L. Siozade, I. Baudin, C. Deumie, K. Glucina, P. Moulin, Membrane characterization by microscopic and scattering methods: multiscale structure, *Membranes* 1 (2011) 91–97, <https://doi.org/10.3390/membranes1020091>.
- [46] Y. Wyart, R. Tamime, L. Siozade, I. Baudin, K. Glucina, C. Deumié, P. Moulin, Morphological analysis of flat and hollow fiber membranes by optical and microscopic methods as a function of the fouling, *J. Membr. Sci.* 472 (2014) 241–250, <https://doi.org/10.1016/j.memsci.2014.08.012>.
- [47] Y. Wyart, S. Nitsche, D. Chaudanson, K. Glucina, P. Moulin, The use of HRSEM to characterize new and aged membranes in drinking water production, *Membr. Water Treat.* 2 (2011) 251–266, <https://doi.org/10.12989/mwt.2011.2.4.251>.
- [48] A. Hart, C. Anumudu, H. Onyeaka, T. Miri, Application of supercritical fluid carbon dioxide in improving food shelf-life and safety by inactivating spores: a review, *J. Food Sci. Technol.* 59 (2022) 417–428, <https://doi.org/10.1007/s13197-021-05022-7>.
- [49] G.C. Soares, D.A. Learmonth, M.C. Vallejo, S.P. Davila, P. González, R.A. Sousa, A. L. Oliveira, Supercritical CO<sub>2</sub> technology: the next standard sterilization technique? *Mater. Sci. Eng. C* 99 (2019) 520–540, <https://doi.org/10.1016/j.msec.2019.01.121>.
- [50] L. Garcia-Gonzalez, A.H. Geeraerd, S. Spilimbergo, K. Elst, L. Van Ginneken, J. Debevere, J.F. Van Impe, F. Devlieghere, High pressure carbon dioxide inactivation of microorganisms in foods: the past, the present and the future, *Int. J. Food Microbiol.* 117 (2007) 1–28, <https://doi.org/10.1016/j.ijfoodmicro.2007.02.018>.
- [51] H.T. Vo, T. Imai, T.T. Ho, M. Sekine, A. Kanno, T. Higuchi, K. Yamamoto, H. Yamamoto, Inactivation effect of pressurized carbon dioxide on bacteriophage Q $\beta$  and  $\Phi$ X174 as a novel disinfectant for water treatment, *J. Environ. Sci.* 26 (2014) 1301–1306, [https://doi.org/10.1016/S1001-0742\(13\)60603-8](https://doi.org/10.1016/S1001-0742(13)60603-8).
- [52] J. Fages, B. Poirier, Y. Barbier, P. Frayssinet, M.L. Joffret, W. Majewski, G. Bonel, D. Larzul, Viral inactivation of human bone tissue using supercritical fluid extraction, *ASAIO J. Am. Soc. Artif. Intern. Organs* 44 (1998) 289–293, <https://doi.org/10.1097/00002480-199807000-00009>.
- [53] A. Checinska, I.A. Fruth, T.L. Green, R.L. Crawford, A.J. Paszczynski, Sterilization of biological pathogens using supercritical fluid carbon dioxide containing water and hydrogen peroxide, *J. Microbiol. Methods* 87 (2011) 70–75, <https://doi.org/10.1016/j.mimet.2011.07.008>.
- [54] A.K. Dillow, F. Dehghani, J.S. Hrkach, N.R. Foster, R. Langer, Bacterial inactivation by using near- and supercritical carbon dioxide, *Proc. Natl. Acad. Sci. USA* 96 (1999) 10344–10348, <https://doi.org/10.1073/pnas.96.18.10344>.
- [55] H.S. Park, Y.H. Lee, W. Kim, H.J. Choi, K.H. Kim, Disinfection of wheat grains contaminated with *Penicillium oxalicum* spores by a supercritical carbon dioxide-water cosolvent system, *Int. J. Food Microbiol.* 156 (2012) 239–244, <https://doi.org/10.1016/j.ijfoodmicro.2012.03.032>.
- [56] M. Kamihiro, T. Asai, Y. Yamagata, M. Taniguchi, T. Kobayashi, Formation of inclusion complexes between cyclodextrins and aromatic compounds under pressurized carbon dioxide, *J. Ferment. Bioeng.* 69 (1990) 350–353, [https://doi.org/10.1016/0922-338X\(90\)90242-O](https://doi.org/10.1016/0922-338X(90)90242-O).
- [57] S.R. Kim, H.J. Park, D.S. Yim, H.T. Kim, I.-G. Choi, K.H. Kim, Analysis of survival rates and cellular fatty acid profiles of *Listeria monocytogenes* treated with supercritical carbon dioxide under the influence of cosolvents, *J. Microbiol. Methods* 75 (2008) 47–54, <https://doi.org/10.1016/j.mimet.2008.04.012>.
- [58] O. Akin, F. Temelli, Effect of supercritical CO<sub>2</sub> pressure on polymer membranes, *J. Membr. Sci.* (2012) 399–400, <https://doi.org/10.1016/j.memsci.2012.01.017>, 1–10.
- [59] S. Milovanovic, M. Stamenic, D. Markovic, J. Ivanovic, I. Zizovic, Supercritical impregnation of cellulose acetate with thymol, *J. Supercrit. Fluids* 97 (2015) 107–115, <https://doi.org/10.1016/j.supflu.2014.11.011>.
- [60] Hans-Georg, Hicke, Ingeburg, Lehmann, Günter, Malsch, Mathias, Ulbricht, Margot, Becker, Preparation and characterization of a novel solvent-resistant and autoclavable polymer membrane, *J. Membr. Sci.*, 198(2), 15 April 2002, Pages 187-196, Doi:10.1016/S0376-7388(01)00595-6.