



HAL
open science

Removal of pathogens by ultrafiltration from sea water

Clémence Cordier, Christophe Stavrakakis, Benjamin Morga, Lionel Degremont, Alexandra Voulgaris, Alessia Bacchi, Patrick Sauvade, Franz Coelho, Philippe Moulin

► To cite this version:

Clémence Cordier, Christophe Stavrakakis, Benjamin Morga, Lionel Degremont, Alexandra Voulgaris, et al.. Removal of pathogens by ultrafiltration from sea water. *Environment International*, 2020, 142, pp.105809. 10.1016/j.envint.2020.105809 . hal-02891935

HAL Id: hal-02891935

<https://hal.science/hal-02891935v1>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Removal of pathogens by ultrafiltration from sea water

Clémence Cordier ^a, Christophe Stavrakakis ^b, Benjamin Morga ^c, Lionel Degrémont ^c,
Alexandra Voulgaris ^b, Alessia Bacchi ^b, Patrick Sauvade ^d, Franz Coelho ^d, Philippe
Moulin ^{b*}

^a Laboratoire de Mécanique, Modélisation et Procédés Propres (M2P2-CNRS-UMR 7340),
Aix-Marseille Université, Europôle de l'Arbois, BP 80, Bat. Laennec, Hall C, 13545 Aix-en-
Provence cedex 04, France

^b Plateforme expérimentale Mollusques Marins, Station Ifremer de Bouin, Polder des Champs,
85230 Bouin

^c Laboratoire de Génétique et de Pathologie des Mollusques Marins, Station Ifremer de La
Tremblade, Avenue du Mus du Loup, 17 390 La Tremblade

^d Suez - Aquasource, 20, avenue Didier Daurat, 31029 Toulouse cedex 04, France

Abstract

Among water treatment processes, ultrafiltration is known to be efficient for the elimination of micro-organisms (bacteria and viruses). In this study, two pathogens were targeted, a bacterium, *Vibrio aestuarianus* and a virus, OsHV-1, with the objective to produce high quality water from seawater. These pathogens are targets, for example, in the case of shellfish productions. The retention of those microorganisms by ultrafiltration was evaluated at lab scale. In the case of OsHV-1, the protection of oysters was validated by *in vivo* experiments using oysters spat and larvae, both stages being highly susceptible to the virus. The oysters raised using contaminated seawater which was then subsequently treated by ultrafiltration, had similar mortality to the negative controls. In the case of *V. aestuarianus*, ultrafiltration allowed a high retention of the bacteria in seawater with concentration below the detection limits of the 3 analytical methods (flow cytometry, direct seeding and seeding after filtration to 0.22 μm). Thus, the quantity of

26 *V. aestuarianus* was at least, 400 times inferior to the threshold known to induce mortalities in
27 oysters. Industrial scale experiment on a several months period confirmed the conclusion
28 obtained at lab scale on the *Vibrio* bacteria retention. Indeed, no bacteria from this genus,
29 potentially harmful for oysters, was detected in permeate and this, whatever the quality of the
30 seawater treated and the bacteria concentration upstream of the membrane. Moreover, the
31 resistance of the process was confirmed with a stability of hydraulic performances over time
32 for two water qualities and even facing an algal bloom. In terms of retention and resistance,
33 ultrafiltration process was validated for the treatment of seawater towards the targeted
34 pathogenic microorganisms, with the aim of biosecuring shellfish productions.

35

36

37 **Keywords:** Ultrafiltration; biosecurisation; pathogen retention; sea water; OsHV-1; shellfish
38 production

39

40 **corresponding author: philippe.moulin@univ-amu.fr*

41 *Tel.: +33 4 42 90 85 01, fax: +33 4 42 90 85 15.*

42

43

44

45

46

47

48

49 I. Introduction

50 In 2014, world oyster production reached the level of 5.2 million tonnes, corresponding to 3.3
51 billion euros (Buestel *et al.* 2009). Since shellfish production is intimately linked to the marine
52 environment, it is therefore sensitive to the events that occur. In the case of the oyster
53 production, the profession is indeed impacted by crises of mortalities, sometimes resulting in a
54 decimation of the breedings as in 1920 and 1971 in France for *Ostrea edulis* and *Crassostrea*
55 *angulata*, respectively. In fact, pathogenic organisms were involved in oyster diseases and
56 massive mortalities. These recurring crises have resulted in the introduction of new and more
57 resistant oyster species. Since the 1970s, oyster *Crassostrea gigas* has been produced almost in
58 monoculture in France. Moreover, oyster producers are using hatchery spat, able to produce
59 diploid and triploid selected oysters for their higher resistance to both diseases in order to
60 supplement or even replace wild-caught spat on oyster farms (Azéma *et al.*, 2016; Burnell and
61 Allan, 2009; Dégremont *et al.*, 2016a; Helm, 2004). Nevertheless, the pollution found in
62 shellfish farms and more generally in aquaculture farms knows(?) various origins and
63 consequences. In hatcheries or nurseries, animals are sensitive to the water quality variations
64 which must yet have characteristics adapted to their growth (Utting and Helm, 1985). In fact
65 microorganisms such as parasites, bacteria, viruses and fungi can be present in feed water at
66 harmful concentrations for aquaculture production (Lekang, 2013). In the case of breeding
67 larval animals, the removal of pathogenic microorganisms is essential at this first stage of life,
68 more vulnerable and susceptible to infections (Azema *et al.* 2017; Degremont *et al.*, 2016)
69 Several virus families have been identified as pathogens in aquaculture, iridoviruses,
70 herpesviruses, reoviruses and rhabdoviruses (Zhang and Gui, 2015). In shellfish production,
71 herpes infections have been reported in nine different species of bivalves (Travers *et al.*, 2015).
72 These pathogens have been associated with significant larval mortalities in hatcheries, in France
73 and New Zealand, and appear to be widespread worldwide. There have always been mortal

74 episodes on *Crassostrea gigas* (*C. gigas*) since its introduction, however, episodes of mortality
75 have been increased on this species since 2008, mainly affecting spat/juveniles due to a
76 pathogenic virus OsHV-1, and affecting adults since 2012 in relation to a pathogenic bacteria
77 *Vibrio aestuarianus* (Azéma *et al.*, 2015; Cochenec *et al.*, 2011; Renault, 2011; Solomieu *et*
78 *al.*, 2015). The shellfish farming profession is also confronted with different families of bacteria
79 known to be pathogenic for animals at various steps of their development (Solomieu *et al.*,
80 2015). Among the pathogenic organisms found, bacteria from the *Vibrio* group, which includes
81 118 species, have been associated with mortalities for many bivalve species (Travers *et al.*,
82 2015). Thus, the oyster mortalities since 2008 in France are mainly linked to the OsHV-1 virus
83 at the spat stage of life and then to specie of *Vibrio* (*aestuarianus*) mainly at the adult stage.
84 The bacterium *Vibrio aestuarianus* was detected in 60 % of the batches of hollow oysters
85 analysed in 2014 and is therefore considered to be one of the most problematic pathogens for
86 the French and European shellfish farming profession (Azéma *et al.*, 2015; Travers *et al.*, 2017).
87 To protect oysters, purification processes can be set up in farms with the objective to deliver
88 water with a constant quality, suitable for farming, respectful of the growth and well-being of
89 animals. For shellfish hatcheries and nurseries, the treatment commonly applied consists of a
90 first filtration step to remove bigger suspended particles and then a UV disinfection step to
91 remove most of the biological organisms. The filtration is essential to avoid an excessive
92 concentration of particles brought to animals, potentially harmful for their growth and ? would
93 favour the appearance of diseases. Processes generally used for the removal of particles are
94 sand filtration or equivalent media, followed by a fine filtration on bags or cartridges to retain
95 particles larger than 10 µm to 1 µm (Ford *et al.*, 2001; Helm, 2004; Wallace *et al.*, 2008). This
96 process does not allow long-term protection of farms, in particular for smaller pollution
97 (pathogenic organisms) and dissolved substances. It must therefore be combined with other
98 processes that is why a disinfection step is needed to inactivate pathogens (Torgersen and

99 Hastein, 1995). The most used processes are first disinfection by ultraviolet (UV) radiations or
100 chemical oxidation processes with chlorine or ozone. In the case of UV disinfection, the dose
101 applied depends on the targeted water quality (targeted pathogens, disinfection objectives) but
102 also on the presence of suspended matter and on the water transmittance. This presence of
103 suspended matter is function of the first filtration treatment efficiency. Many advantages are
104 specific to UV, such as in situ generation, therefore no storage of toxic or dangerous products,
105 no risk of overdose and very fast action (Summerfelt, 2003). Moreover, unlike the membrane
106 filtration, UV radiation is not able to remove bacteria and virus, it only inactivates them.
107 Regarding pathogenic organisms, this process has been shown to be effective for the
108 inactivation of the OsHV-1 virus and the bacteria *Vibrio aestuarianus* by Stavrakakis *et al.*
109 (2017). A dose of 50 mJ cm⁻² is thus effective using a low-pressure UV system for both
110 pathogen inactivation. Among the drawbacks of water treatment with UV radiation, the aging
111 of the lamps, the revival of some microorganisms and the influence of the quality of the water
112 (transmittance) to be treated on disinfection performances, can be mentioned (Gullian *et al.*,
113 2012; Martínez *et al.*, 2013; Qualls and Johnson, 1983). The previous step of removing
114 suspended particles is then also necessary to guarantee the effectiveness of UV treatments that
115 could be impacted by the presence of particles (Lekang, 2013). In addition, the generation of
116 degradation by-products during the treatment of chemical molecules by UV was highlighted
117 (Souissi *et al.*, 2013). Ozone, the most powerful industrial oxidant, is used in many applications
118 for its oxidizing and disinfecting capacities. Thus, reducing the bacterial load or improving the
119 yield and production growth are beneficial effects of the use of ozone promoted by Powell *et*
120 *al.* (2016) on the cultivation of crustaceans and molluscs. In oyster farming, the effectiveness
121 of this process has been demonstrated for specific pathogens, the herpes virus OsHV-1 and the
122 bacterium *Vibrio aestuarianus*. A treatment of 30 min at a concentration of 1 mg L⁻¹ is effective
123 for the protection of bivalves (Stavrakakis *et al.*, 2017). Ozone can also be coupled with UV

124 radiation to improve treatment. Sharrer *et al.* (2007) showed that this combination resulted in
125 almost complete inactivation in coliform and heterotopic bacteria in fresh water (42.5–
126 112.7 mJ cm⁻²). Ozone, however, has drawbacks too: its production cost and the fact that this
127 oxidant is toxic towards humans and animals (Lekang, 2013; Moretti *et al.*, 1999). Powell *et al.*
128 (2016) have highlighted a negative impact on hatching rates, growth, until mortality
129 observations on shellfish. Finally, chemical oxidation leads to the formation of oxidation
130 residues and disinfection by-products which can impact the species living in the natural
131 environment, or farms in the case of a closed circuit (Delacroix *et al.*, 2013; Kornmueller, 2007;
132 Lazarova *et al.*, 1999; Richardson *et al.*, 2007).

133 In this paper, ultrafiltration (UF) is evaluated on its capacity to produce a high-quality water for
134 bio-securisation. In fact, this process well used in other industrial application, especially to treat
135 seawater, allow a purification by a steric effect without drawbacks on the quality of the water
136 produced (Greenlee *et al.*, 2009; Karakulski *et al.*, 2002; Wolf *et al.*, 2005). For the first time,
137 this innovative process is used to bio-secure shellfish farms against specific pathogens. The first
138 part focuses on the specific removal of two pathogens: the herpes virus OsHV-1, and the
139 bacterium *Vibrio aestuarianus*, known for their impact on shellfish production. In a second part,
140 the aim is to evaluate over the long term (8 months), and at an industrial scale, the performances
141 of the process for the abatement of the bacteria load present in the sea water feeding the process
142 (total flora and *Vibrio* bacteria). The UF pilot used in this work was studied in terms of retention
143 and hydraulic performance, monitored on different feed water qualities.

144

145 **II. Materials and Methods**

146 **II.1 Semi-industrial and lab scale ultrafiltration pilot plants**

147 The ultrafiltration membrane used for the experiments were hollow fibres (ALTEONTM I ;
148 SUEZ environnement-Aquasource, France) in polyethersulfone with 7 channels of a 0.9 mm

149 inside diameter and 1.2 m long. Their MWCO was $0.02 \mu\text{m}$ and initial permeability 1000 L h^{-1}
150 $\text{m}^{-2} \text{ bar}^{-1}$ with a 8 m^2 of membrane surface (Cordier *et al.*, 2019a,b, 2018). The semi-industrial
151 pilot was able to treat $20 \text{ m}^3 \text{ d}^{-1}$. The tests were all performed in dead end filtration. Hydraulic
152 performances, L_p and TMP, respectively membrane permeability and transmembrane pressure,
153 were registered every minute by the pilot system. Turbidity was measured and recorded every
154 minute in the feeding tank of the UF pilot using a prob VisoTurb 700 IQ (WTW). In order to
155 maintain a flux productivity and to sustain an efficient process, a frequent chemical cleaning
156 (CEB) was conducted during this investigation. Chemical cleaning (CEB) was a two steps
157 procedure: first a basic solution with an addition of chlorine ($\text{pH} = 9.5$) in order to reach a
158 chlorine concentration between 100 and 200 ppm in membranes depending on the treatment
159 needed, was injected in the membranes. Then, after 30 min, the module was rinsed with
160 permeate at $2 \text{ m}^3 \cdot \text{h}^{-1}$ and filled with an acid solution ($\text{pH} = 2$). After 30 min the module was
161 finally rinsed with permeate at $2 \text{ m}^3 \cdot \text{h}^{-1}$. The limit of permeability before chemical cleaning was
162 $250 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$.

163 For confinement constraints, a lab scale pilot was used with the same hollow fiber membranes.
164 The membrane module, with an active area of 0.138 m^2 , was able to treat a volume of 8.5 L.
165 These characteristics allow a volumic concentration factor (VCF) of 267, similar to the semi-
166 industrial pilot. Hydraulic performances, L_p and TMP were collected by weighting the
167 permeate every minute and following the pressure gauge. The transmembrane pressure applied
168 for the ultrafiltration treatment was 0.3 bar. All the results are expressed taking into account the
169 variation of temperature.

170

171 **II.2 OsHV-1**

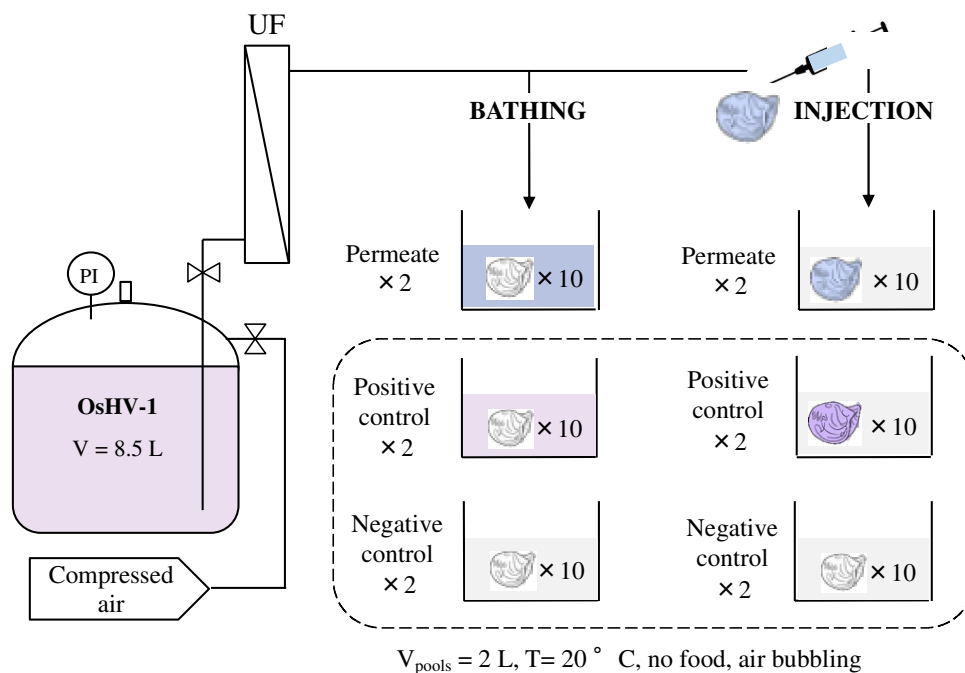
172 **II.2.1 Oysters and contaminated sea water**

173 Two groups of oysters were used during the experiments: oysters selected for their higher
174 susceptibility to OsHV-1 and *V. aestuarianus* according to Azéma *et al.* (2017) and unselected
175 oysters (NSI) produced according to a standardized protocol describe by Petton *et al.* (2015).
176 The selected oysters were used to prepare the contaminated sea water (*C. gigas* oysters selected
177 for their high sensitivity to the OsHV-1 virus, age < 1 year, length = 2 cm, width = 1 cm,
178 experimental oysters produced by Ifremer). Meanwhile, NSI which means standard oyster spat
179 Ifremer, were used to be maintained in the ultrafiltered seawater in order to verify the virus
180 retention. The contaminated solution was prepared by injecting a viral solution into the adductor
181 muscle of the oyster. To perform the injection into the muscle, the oysters must be anesthetized.
182 For this, the oysters were placed dry for 4 hours before falling asleep. These stressful conditions
183 encourage them to open more quickly and filter as soon as they are returned to water. The
184 oysters were anesthetized (T = 20°C) in hexahydrate MgCl₂ (50 g L⁻¹) for 2 h (Suquet *et al.*,
185 2009). Then, they were injected with 10 µL of viral solution in the adductor muscle to allow
186 spreading into the circulatory system. The OsHV-1 inoculum was prepared and produced
187 according to Schikorski *et al.* (2011), and injections were performed 24 h before the start of the
188 experiment. Then, oysters were placed in disinfected seawater (T = 20 °C) during 24 h. The
189 conditions of the 3 tests, number of oysters and seawater volume used to prepare contaminated
190 sea water, are 85/20, 200/25 and 200/25 oysters L⁻¹ respectively for test 1, 2 and 3 (Morga *et*
191 *al.*, 2020). After 24 h, the sick oysters released the virus in the seawater: a contaminated
192 seawater is obtained to be treated by ultrafiltration. The transmembrane pressure applied for the
193 ultrafiltration treatment was 0.3 bar. Permeate weight was collected each minute in order to
194 determinate flowrate and membrane permeability.

195

196 **II.2.2. Evaluation of virus retention**

197 Virus retention was evaluated by two methods as describe in Figure 1: bathing and injection.
 198 For bathing, 10 × 2 spat oysters were placed into 3 × 2 pools filled with 2 L of permeate,
 199 disinfected seawater by ultrafiltration (negative control) and contaminated seawater (positive
 200 control). For the injection method, 10 × 2 spat oysters were injected with 100 μL of permeate,
 201 disinfected seawater (negative control) and contaminated seawater (positive control) containing
 202 virus. Injected oysters were then placed in 6 pools of disinfected seawater at 20 °C. Negative
 203 controls enable to verify that oysters could face experiment conditions. Meanwhile, positive
 204 controls, which were the oysters in contact with contaminated seawater, verified the
 205 pathogenicity of the OsHV-1. Each test was duplicated in order to have 2 negative controls, 2
 206 positive controls and 2 permeates in bathing and injection evaluation. For each condition,
 207 cumulative mortality was monitored every 24 h for 7 days.



208
 209 *Figure 1: Treatment of OsHV-1 – In vivo test of bathing and injection – Pink: contaminated solution*
 210 *with OsHV-1; blue : treated water (=permeate) and grey: disinfected seawater [TMP = 0.3 bar]*

211

212 **II.2.3. OsHV-1 analysis larvae mortality data analyses**

213 For the larval experiment, mortality was analyzed by a binomial logistic regression throughout
214 the GLIMMIX procedure (SAS® 9.4 software, Cary, NC, USA) at day 3 and day 7 post-
215 infection according to the following model:

$$216 \text{logit}(\pi_{ij}) = \mu + \text{condition}_i + \text{replicate}_{(ji)}$$

217 where π_{ij} is the probability of the mortality at day 3 or day 7 for oyster of the “i” condition
218 (negative control, positive control, and permeate)(fixed factor) for the “j” replicates (6
219 wells)(random factor) and μ the intercept.

220

221 **II.2.4. Polymerase chain reaction (QPCR) for virus analyses**

222 Different seawater samples were collected during the filtration step: first and last millilitre of
223 permeate, retentate and the inlet contaminated seawater (Table 1). A dead-end filtration is
224 operated, so the permeate is divided into two identical volumes P1 and P2 to take into account
225 the increased concentration (i.e. the membrane retention) in the hollow fibre lumen. Dead
226 oysters were also collected to be analysed. Each sample was analysed by QPCR at Ifremer
227 Laboratory of Genetics and Pathology of Marine Molluscs (LGPMM, La Tremblade, France).
228 The QPCR limit detection was estimated at 10 copies of virus DNA per microliters of samples
229 (Pepin *et al.*, 2008).

230

231 **II.3 *Vibrio aestuarianus***

232 **II.3.1. Contaminated sea water**

233 *Vibrio aestuarianus* GFP strain was provided in Petri dish by Ifremer LGPMM (La Tremblade,
234 France). The *Vibrio aestuarianus* GFP is a modified strain that gets kanamycine resistant and
235 fluorescent characteristics. The bacteria solution was prepared 24 h before the ultrafiltration
236 tests at a 10^9 CFU L⁻¹ concentration following the protocol developed by (Azéma *et al.*, 2016).

237 After 24 h, the bacteria solution was then seeded in a 10 L autoclaved seawater to produce a
238 contaminated seawater at 10^6 CFU L⁻¹. 8.5 L of contaminated seawater was ultrafiltrated by the
239 lab scale pilot. Permeate weight was collected each minute in order to determinate flowrate and
240 membrane permeability. Performances of bacteria removal by ultrafiltration was determined by
241 cytometry and specific bacteria analysis.

242

243 **II.3.2. Cytometry and bacteria analysis**

244 Due to its fluorescent characteristics *Vibrio aestuarianus* GFP is detectable by cytometry
245 analysis. 800 µL of contaminated seawater, permeate, concentrate and autoclaved seawater
246 were analysed in triplicate by flow cytometry (CyFlow-Partec-Sysmex) after 4 min with a
247 threshold fixed on FL1 fluorescence (Aboubaker *et al.*, 2013; Travers *et al.*, 2017). Beside flow
248 cytometry, two types of bacteria analysis were carried out: bacterial analysis on Petri dishes
249 and bacteria analysis with a vacuum filtration on 0.2 µm cellulose filter before growth on Petri
250 dishes. *Vibrio aestuarianus* analysis were carried out in salted luria broth agar medium with
251 kanamycine which allows only *Vibrio aestuarianus* GFP growth. For the bacteria analysis,
252 50 µL of sample was deposited on the Petri dishes and then incubated at 20 °C during 48 h. A
253 second bacteria analysis method was performed by filtering 500 mL of sample on the cellulose
254 filter, then the filter was deposited on Petri dish and incubated at 20 °C during 48 h.

255

256 **II.3.3. Microbiologic analyses**

257 In shellfish culture, some species of *Vibrio* being pathogenic for oysters, their presence in the
258 water supplying spat and larvae are also monitored. Total bacterial load and *Vibrio* were
259 analysed in these waters by microbiologic analyses realised twice a week in the pilot feed and
260 in permeate (both at the beginning and the end of the filtration cycle). The aim of these analyses
261 was to verify the absence of *Vibrio* bacteria but also to estimate the total bacterial retention by

262 UF. *Vibrio* analysis were carried out in TCBS agar medium and total bacterial load on marine
 263 agar medium (Aboubaker *et al.*, 2013). 50 μ L of each water sample was deposited on the Petri
 264 dishes and then incubated at 20 °C during 48 h. Retentions at the beginning and end of the
 265 filtration cycle are calculated from the equations below:

$$\text{Initial retention} = \frac{C_{feed} - C_{first\ mL}}{C_{feed}} \quad \text{Final retention} = \frac{C_{retentate} - C_{last\ mL}}{C_{retentate}}$$

266 with: C_{feed} : virus concentration in the feed, $C_{retentate}$: virus concentration in the retentate, C_{first}
 267 mL: viral DNA concentration in the first mL of permeate and $C_{last\ mL}$: viral DNA concentration
 268 in the last mL.
 269

270

271 *Table 1: Treatment of OsHV-1 – Synthesis of analyses realized on permeates obtained*

Permeate	First mL	P1	P2	$P_{average}$ = P1 + P2	Last mL
Volume recovered (L)	0.05	4.2	4.2	= 4.2 + 4.2	0.05
In vitro analyses	×	×	×	×	×
In vivo analyses				×	
Total bacteria and <i>Vibrio</i> measurement	×			×	×

272

273 **III. Results and discussion**

274 In this paper, ultrafiltration is evaluated on its capacity to biosecure shellfish farms and to make
 275 oyster farming more sustainable. The aim is the treatment of feed sea water for the protection
 276 of larvae and spat against pathogenic organisms. The retention and hydrodynamic
 277 performances, as well as the resistance of the process to the fouling generated on the membrane
 278 in the case of these particular pollutions, are monitored.

279

280 **III.1. Specific tests for *C. gigas* pathogen retention: laboratory scale**

281 The tests were carried out using a laboratory scale ultrafiltration device to meet the confinement
 282 constraints linked to working with sensitive bacteria and viruses on a production site. This
 283 device is equipped with the same membranes as the industrial-scale pilot (used for the long-
 284 time evaluation) and operates at constant pressure. The volumic concentration factor (VCF) of
 285 the industrial pilot is the same on the laboratory pilot by choosing a filtering surface of 0.13 m²
 286 and a volume of solution to be treated of 8.5 L, for J60 t60 conditions (i.e. Permeate flux J =
 287 60 L h⁻¹ m⁻² and filtration time t = 60 min). In addition, the ultrafiltration tests are carried out
 288 at a transmembrane pressure (TMP) of 0.3 bar which corresponds to the maximum TMP
 289 reached during the filtration of seawater on the industrial-size pilot under similar J60 t60
 290 conditions. Whether for OsHV-1 and *V. aestuarianus*, the general protocol followed was
 291 identical and took place in three stages: (i) preparation of a contaminated sea water, (ii)
 292 ultrafiltration of this solution with flow monitoring and (iii) verification of performance by *in*
 293 *vivo* and / or *in vitro* tests with positive and negative controls.

294

295 III.1.1. Virus OsHV-1 treatment: Virus retention

296 Three experiments were carried out on the virus. The characteristics of the experiments, initial
 297 permeability measured with demineralized water, filtration time, turbidity, DNA concentration
 298 of OsHV-1 and total flora of the contaminated sea water are summarized in Table 2.

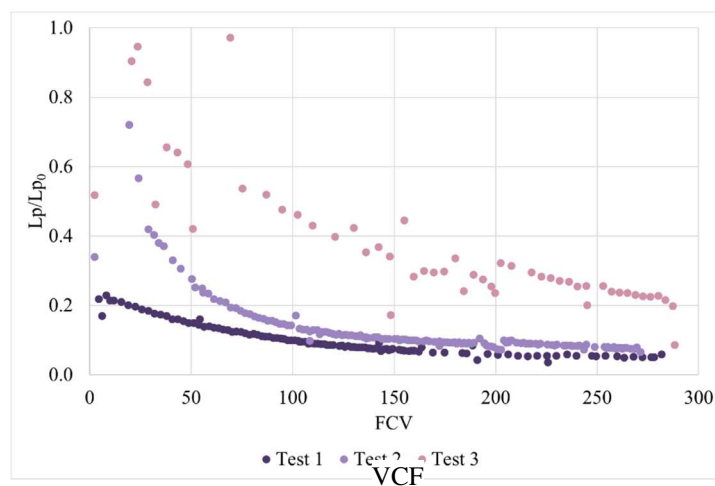
299

Table 2: Characteristics of OsHV-1 experiments

Test	Filtration		Turbidity (NTU)	Contaminated seawater		
	Lp ₀ (L h ⁻¹ m ⁻² bar ⁻¹)	Filtration time (min)		Number of oysters/Seawater Volume L	OsHV-1 Concentration (DNA copies μL ⁻¹)	Total bacteria (CFU mL ⁻¹)
1	780	210	5.5	85 / 20	8.7.10 ²	4.39.10 ⁶
2	607	167	8.2	200 / 25	9.1.10 ¹	8.79.10 ⁶
3	770	47	4.6	200 / 25	4.2.10 ²	1.19.10 ⁶

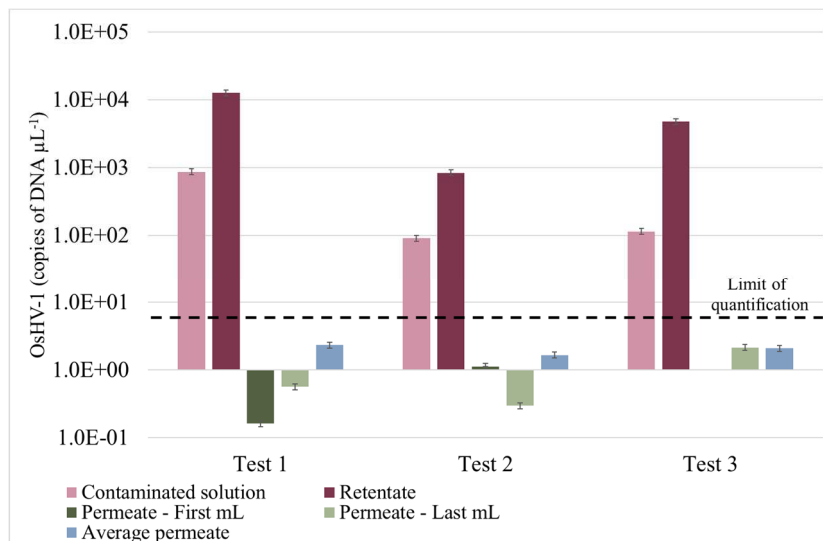
300 In the test 1, the virus concentration was judged too low. In order to work with a contaminated
 301 solution with a higher OsHV-1 DNA concentration, the number of oysters used for the
 302 preparation of the solutions for tests 2 and 3 was increased from 85 to 200 oysters (Table 2).

303 However, contrary to what was expected, the increase of the viral concentration in the solutions
 304 was not significant. In the case of test 2, it was even lower than test 1 with less than 10^2 copies
 305 of DNA μL^{-1} in the contaminated solution. This result could be explained by a reduced
 306 susceptibility of the oysters in tests 2 and 3, because even if they were from the same batch,
 307 they were older (3 months) and larger than in test 1, as demonstrated by Dégremont (2013).
 308 24 h post-injection, mortalities were observed among the animals used for the preparation of
 309 the contaminated seawater by OsHV-1 dedicated to the tests 1 and 2. Those dead oysters
 310 generated a viscous deposit in the solution obtained testifying to a more turbid and more loaded
 311 bacteria solutions than for test 3, especially for the test 2 as shown by the variation in filtration
 312 time? As the variation in filtration time shows. The variation of the permeability as a function
 313 of the VCF is presented in Figure 2 for the 3 tests carried out. The differences of the number of
 314 dead oysters to prepare solution and the difference of turbidity, virus and bacterial
 315 concentration, led to different fouling generated by the filtration of the 3 solutions obtained.
 316 Moreover, the effect of these initial parameters is accentuated by the concentration factor in the
 317 membrane. Membrane fouling seems to be linked more to parameters such as suspended matter
 318 (turbidity) and the presence of bacteria than to the concentration of OsHV-1 in the seawater, it
 319 is in agreement with the pore size. In all 3 cases, the permeability loss remains reasonable with
 320 final permeate fluxes around $100 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$ from high concentrated solutions.



321
 322 *Figure 2: Evolution of permeability vs. VCF – Retention of OsHV-1*

323 The retention performance of the virus is controlled by measurements of the concentration of
 324 OsHV-1 viral DNA by qPCR analyses in the contaminated water, in the retentate and permeate.
 325 Several samples of permeate are analyzed: first mL, last mL, first 4.2 liters, last 4.2 liters and
 326 average permeate in order to take into account the variation of the concentration inside the
 327 membrane during the experiment. The concentrations measured on these different samples and
 328 for the 3 tests are presented Figure 3. Whatever the viral DNA concentration in the initial
 329 solution, if virus is detected in the permeates, the measured value is always less than the qPCR
 330 quantification limit (10 copies of viral DNA μL^{-1}). The analyses revealed very low amount of
 331 virus, not quantifiable, in the permeate samples.



332

333 *Figure 3: DNA of OsHV-1 concentrations in samples after ultrafiltration of contaminated solution -*
 334 *qPCR analysis*
 335

336 Retentions at the beginning and end of the filtration cycle are calculated as previously
 337 explained, for the different experiments and presented in Figure 4. To be noted that, when the
 338 concentration measured is inferior to the quantification limit, the value used for retention
 339 calculation is this threshold, 10 DNA copies μL^{-1} .

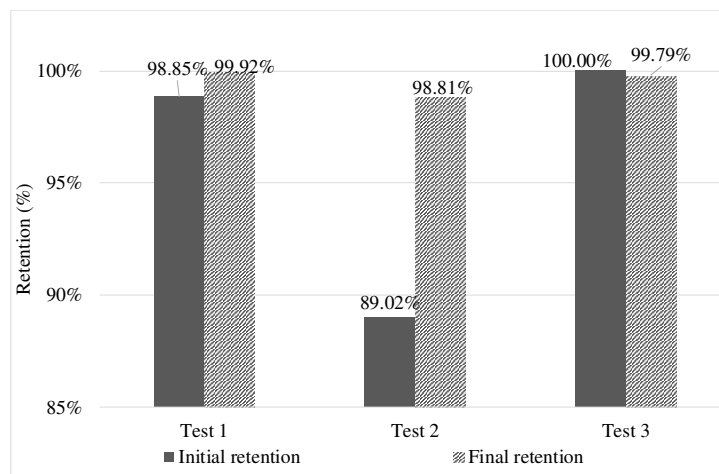
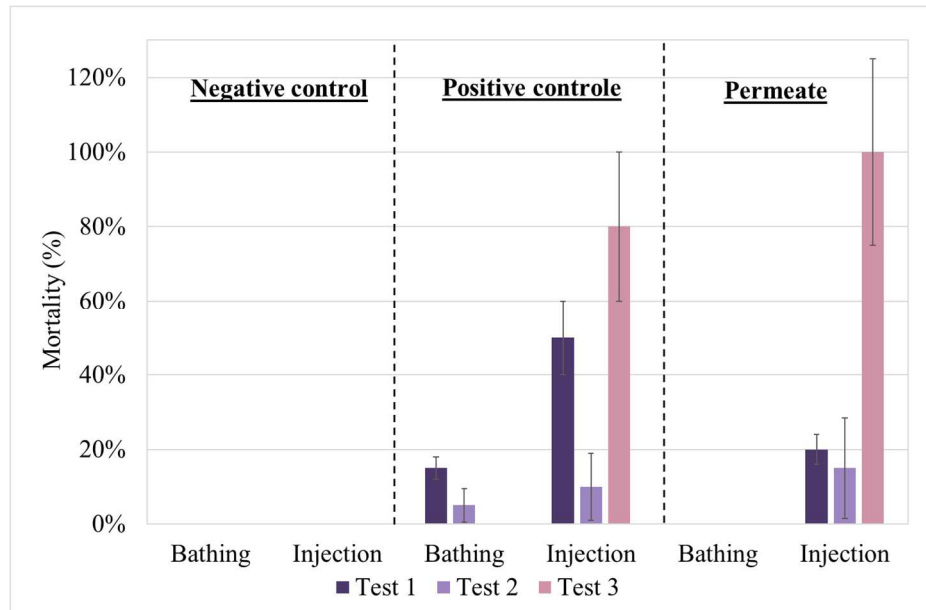


Figure 4: *OsHV-1* retentions at the beginning and end of filtration cycle for the 3 tests

340
 341
 342
 343 Retention was always greater than 89 % and retention of 100 % was even achieved for test 3
 344 since the virus was not detected in the first mL of permeate. The minimum value of 89 % may
 345 appear low but it is important to specify that the retention values are calculated using for the
 346 limit of quantification of 10 copies of viral DNA μL^{-1} , leading to an underestimation. This
 347 underestimation is even more pronounced when the concentration in the feed is low: test 2
 348 showed the lower retention because of a concentration in the contaminated solution of
 349 100 copies of DNA μL^{-1} . If the virus concentrations were below the QPCR quantification limit
 350 in the 3 tests, the retention was not total since the presence of DNA from the virus is almost
 351 always detected in the permeate. Two questions arise following these results: (i) is the amount
 352 of virus still present in the permeate sufficient to induce oyster mortality? and (ii) does the DNA
 353 detected, once it has passed through the pores of the membrane (20 nm), belong to a virus able
 354 to infect oysters? Indeed, the QPCR carried out makes it possible to detect traces of viral DNA
 355 but does not specify whether this DNA is from an integrated viral particle. To answer these
 356 questions and to conclude on the performances of the UF process for the removal of this
 357 pathogenic agent, *in vivo* tests were performed. Two tests were set up to assess the infectiosity
 358 of the treated solution: bathing of oyster spat (n = 10, V= 2 L of water, in duplicate) and larvae
 359 (n = 150 larvae per 6 mL well, 6 wells per condition), and injections in spat (10 individuals

360 injected in duplicate). To validate the results obtained, the same manipulations were each time
361 carried out on positive and negative controls, under the same conditions. The mortalities
362 obtained after 7 days of contact, for the immersion (or bath) and oyster injection tests on the 3
363 tests, are presented in Figure 5. The dead oysters were frozen, and their flesh was then analyzed
364 by QPCR. In all the samples analyzed, a concentration greater than or equal to 10^3 copies of
365 viral DNA μL^{-1} was measured, which confirms that all the oysters died due to a viral infection.
366 Regarding the controls, as expected, (a) no mortality in the negative controls, either by injection
367 or by bathing was observed. The oysters were not affected by an injection of $100 \mu\text{L}$ (Schikorski
368 *et al.*, 2011) and withstood the conditions of the experiment at $20 \text{ }^\circ\text{C}$ in ultrafiltered seawater
369 without food during the 7 days of follow-up; (b) mortalities were observed for positive injection
370 and bathing controls. As expected, the mortalities by injection are higher than those in bathing
371 for each test. These results are consistent since, during the injection, the virus enters the oyster's
372 body directly without going through the filtration and incorporation stage, as in the case where
373 the virus is found in the bathing water (Schikorski *et al.*, 2011). In experiment 2, low mortality
374 was observed in the positive injection controls which could be explained by their age, close to
375 18 months, making the oysters more resistant to the virus. For experiment 3, the tests were
376 therefore carried out on the selected oysters for their higher susceptibility to the virus, and the
377 average mortality was 80 % in the positive controls by injection. However, on this same
378 experiment, no mortality was observed in bathing oysters, so no conclusion can be drawn.



379

380 *Figure 5: In vivo tests results – Oyster spat mortalities after 7 days in contact by bathing and injection*
 381 *with negative, positive controls and permeate*
 382

383 Concerning the permeate, 100 % mortality was obtained on the injected oysters but none for
 384 those in bathing. These results are correlated with QPCR analyzes: the viral DNA is detected
 385 in low concentration, sufficient to kill oysters when the permeate is injected directly inside the
 386 body, but insufficient to kill oysters in bathing over 7 days. Ultrafiltration provides protection
 387 of oysters at the spat stage when the permeate is in bathing contact with the oysters, closer to
 388 real production conditions. In order to validate the effectiveness of ultrafiltration for the
 389 protection of shellfish farms, during experiment 3, the immersion or bath tests were carried out
 390 with oyster larvae 8 days old, life stage more sensitive than spat previously used (Degrémont,
 391 Morga *et al.* 2016). As for spat, the larvae were brought into contact with the permeate produced
 392 and the cumulative mortalities at days 3 and 7 were compared with those obtained for bathing
 393 larvae in contaminated water and ultrafiltered seawater. At day 3, mortality was not
 394 significantly different among conditions ($P = 0.48$) with 23 %, 25 %, and 36 % for the negative
 395 control, the permeate, and the positive control, respectively. P value lesser than 0.05 indicates
 396 strong evidence against the null hypothesis which was the absence of effect on larval mortality
 397 among conditions (ultrafiltration vs negative vs positive controls). At day 7, larvae of the

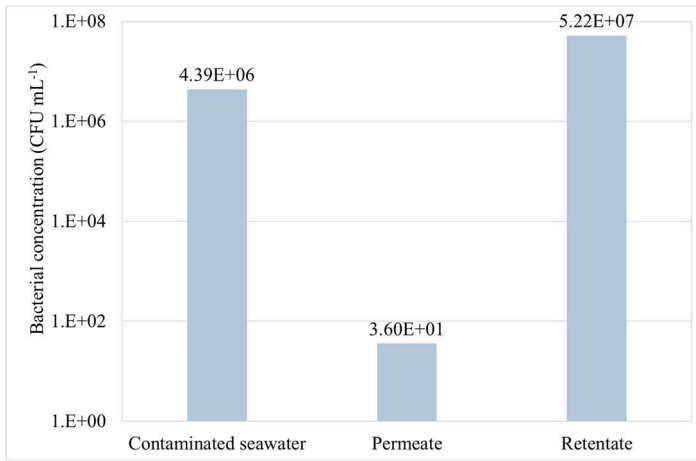
398 positive control showed a significant higher mortality (100 %) than those of the negative control
399 (44 %) and the permeate (56 %) ($P < 0.01$). Although the mortality of the larvae in contact with
400 the permeate was 12 % higher than in the negative control, this difference was not significant
401 ($P = 0.23$). Thus, it appears that the protection of the larvae by the ultrafiltration process was
402 effective against OsHV-1. Mortality observed for the negative controls, as well as the permeate
403 condition could be both related to physiological-genetic characteristics and the conditions of
404 the 6-well plates experiment that lasted 7 days without feeding. Thus, it is common to observe
405 some mortality for D larvae, as some of them will be unable to survive (abnormal shape,
406 deleterious/lethal genes..), as well as those near the metamorphosis (Dégremont *et al.*, 2016b).

407

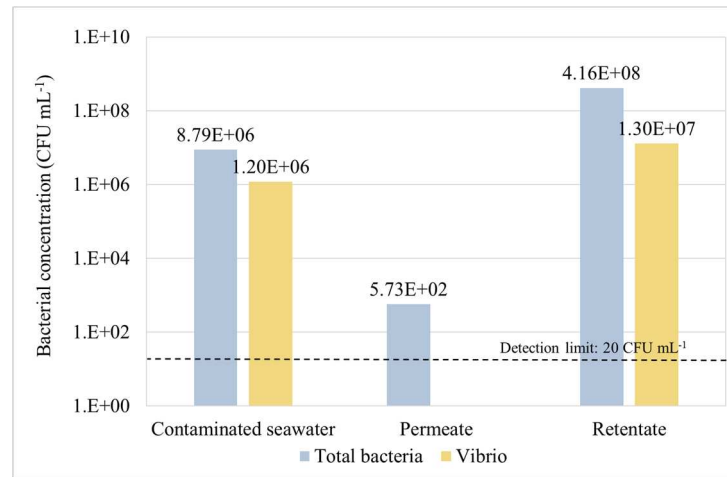
408 **III.1.2. Virus OsHV-1 treatment: Bacteria retention**

409 Bacterial removal was measured on total flora and *Vibrio* bacteria, present in contaminated
410 seawater by OsHV-1. The results put in light a reduction of at least 4 logs of the total flora in
411 each experiment by ultrafiltration (Figure 6). The *Vibrio* concentrations from Petri dishes in
412 experiment 1 could not be determined because of the too large number of colonies formed on
413 agar media (> 300 CFU). Dilutions were made for the following experiments to facilitate the
414 enumeration. The retention of bacteria of the *Vibrio* genus obtained for experiments 2 and 3
415 was high since no colony was detected on the petri dishes corresponding to the permeates
416 analyzed and this for different initial bacterial concentrations.

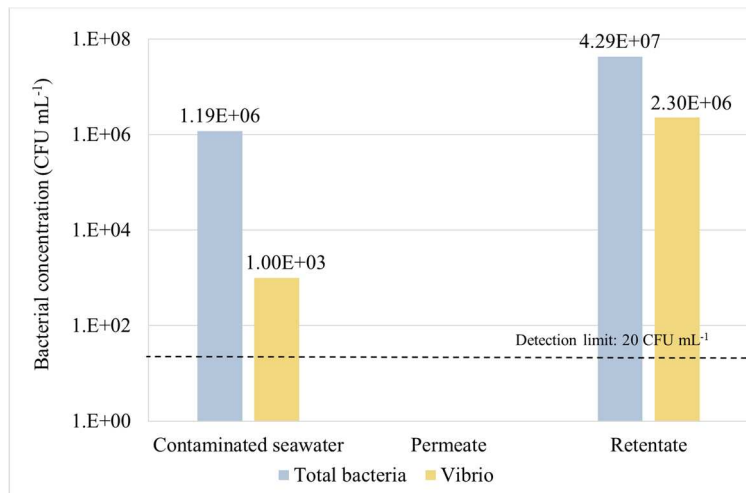
417



Test 1



Test 2



Test 3

Figure 6: Total bacteria and *Vibrio* bacteria concentrations

418

419

420

421

422 The initial and final retentions of total flora and bacteria (*Vibrio* genus) were determined for

423 each experiment (Table 3), showing the high performances of the membrane to reduce the

424 bacteria load, with removal from 4.19 to more than 6.33 log and from 2.99 to more than 7.11 log

425 for total bacteria and *Vibrio* bacteria respectively.

426

427

428

429

Table 3: Initial and final removal of bacteria obtained during OsHV-1 experiments

Test	Total bacteria		<i>Vibrio</i>	
	Initial removal (Log)	Final removal (Log)	Initial removal (Log)	Final removal (Log)
1	5.08	6.16	-	-
2	4.19	5.86	> 6.08	> 7.11
3	> 4.77	> 6.33	> 2.99	> 6.36

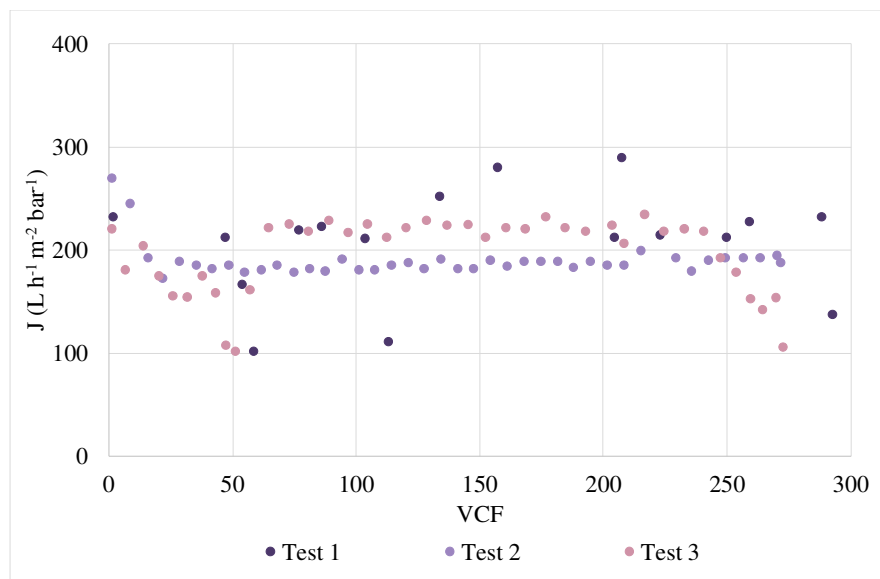
430

431 As conclusion, the tests carried out with OsHV-1 virus highlight a retention of this compound
432 by ultrafiltration membranes with values higher than 4 log which is the retention given by the
433 membrane manufacturer for bacteriophage MS2, virus with a lower size (25 nm) than OsHV-
434 1. However, this retention is not total because traces, not quantifiable, are detected by QPCR in
435 the permeate. This result is confirmed by *in vivo* tests: when the permeate obtained was injected
436 into oysters, mortalities comparable to those obtained for positive controls were noted at the
437 spat stage. The presence of viruses in the permeate despite the pore size of the membranes could
438 be justified by an heterogeneity of the pores of this sample of membranes or by the
439 implementation (or manufacturing) of the micro-modules used for these tests. If the injection
440 test validates the presence of the virus in the permeate and its retained infectiosity, it does not
441 reflect actual hatchery conditions. The balneation tests then carried out, in real conditions,
442 underline a protection of the oysters by the process since no bathing mortality was observed on
443 the 3 tests after ultrafiltration. This result was confirmed with bathing larvae, the most
444 susceptible stage of the oyster for OsHV-1. Moreover, it is important to note, that we have used
445 harsh conditions and in reality, the feed water viral concentrations is not at this level used for
446 these experiments. In addition, the bacteriological analyzes carried out underline the
447 performance of the ultrafiltration for the reduction of the total flora (> 4 log) and particularly
448 bacteria of the *Vibrio* type (> 7 log with no detection in the permeate), which represents
449 preliminary results encouraging for specific tests on *Vibrio aestuarianus* but also for long-term
450 follow-up tests for retention of total *Vibrio*.

451

452 **III.1.3. Retention of the *Vibrio aestuarianus* bacteria**

453 The tests were carried out on a strain of *Vibrio aestuarianus* GFP, modified to be detectable by
454 flow cytometry without the addition of a marker and resistant to an antibiotic, kanamycin
455 (which makes it possible to obtain cultures specific to this bacterium). Three filtration tests,
456 were carried out with a theoretical bacterial concentration in the initial solution of 10^5 - 10^5 and
457 10^6 CFU mL⁻¹. The variation of flux as the function of the VCF is presented Figure 7. A more
458 moderate fouling than in the case of the treatment of viral solutions was observed. This result
459 is explained by the quality of the matrix containing the microorganisms: in the case of the virus
460 the solution was prepared from oysters which also brought organic matter (bacteria, faeces,
461 rotting flesh) to the solution in addition to the virus, which is not the case for *Vibrio*
462 *aestuarianus* bacteria solutions.



463
464 *Figure 7: Evolution of flux vs. VCF for Vibrio aestuarianus retention tests*

465
466 (i) Monitoring of retention performance by seeding on a Petri dish

467 Two types of sample seeding were carried out: with filtration on a cellulose filter (0.22 μ m) and
468 without filtration. Only the results of tests 2 and 3 are presented in Figure 8a, because an
469 external contamination appeared for the test 1. The formation of a circle concentrated in bacteria

470 in the center of the filter was observed for the contaminated seawater which was not the case
471 for the medium permeate and the negative control. The appearance of mold on the filters in test
472 3 both in permeate and negative control showed that the filtration was carried out under non-
473 sterile conditions. However, the specific and selective medium used, LBS + kanamycin, limit
474 the appearance of bacteria originating from handling under non-sterile conditions. The
475 advantage of the filtration method is the concentration of bacteria in a sample containing a very
476 small quantity leading to a detection limit of 2 CFU L⁻¹ versus 20 CFU mL⁻¹ in the case of direct
477 seeding without filtration. Thus, the 500 mL of first and last 4.2 L of permeate and average
478 permeate (which is the mix of the two permeates P1 and P2 obtained), were filtered in triplicate
479 and no bacteria was observed on the 9 filters. The removal rate cannot be calculated with this
480 method because the contaminated seawater is too concentrated to allow a count of the colonies
481 formed. However, the absence of a colony in the permeate indicates high retention, the limit of
482 detection (2 CFU L⁻¹) is therefore given for manipulations 2 and 3 as the maximum value. With
483 the direct seeding method, no colony was observed in the permeates (first and last 4.2 L of
484 permeates (P2) and average permeate (P1+P2)). The initial and final retentions calculated for
485 the 3 experiments are reported Figure 8b. The difference between the initial and final removal
486 is consistent with the VCF, reflecting an increase in the concentration upstream due to filtration
487 and a zero concentration downstream from the start to the end of the experience. Ultrafiltration
488 allowed total retention of the *Vibrio aestuarianus* bacteria with minimum abatements between
489 5 and 7 log.

490

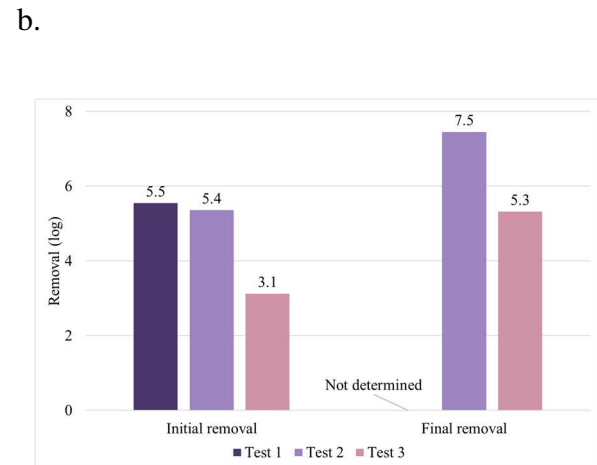
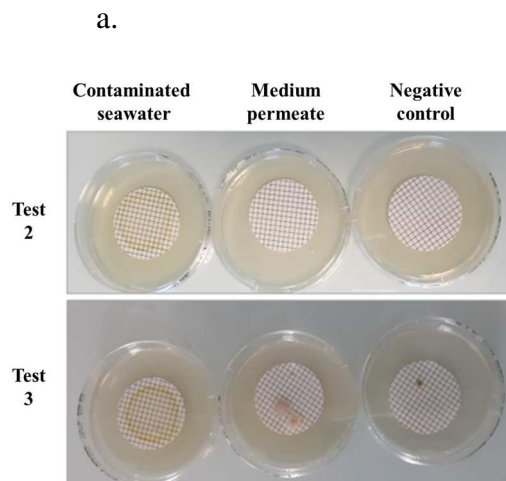
491

492

493

494

495



496 *Figure 8: Treatment of Vibrio aestuarianus - a. Pictures of filters 48 h after incubation*
 497 *and b. Removal of Vibrio aestuarianus calculated from direct seeding results – Seeding*
 498

499 (ii) Monitoring of retention performance by flow cytometry

500 In order to validate the retention performance, the samples (contaminated solution, medium
 501 permeate, retentate, autoclaved seawater) were also analyzed by flow cytometry. The results
 502 are reported in Figure 9. For all the permeates analyzed for the 3 tests, the concentration
 503 measured was below the detection limit (10^2 CFU mL⁻¹). In the case of tests 1 and 2, a second
 504 population was observed outside of zone R2 but this new population comes from ultrafiltered
 505 and autoclaved seawater used to prepare the bacterial solutions in agreement with the cytograms
 506 of the negative control.

507

508

509

510

511

512

513

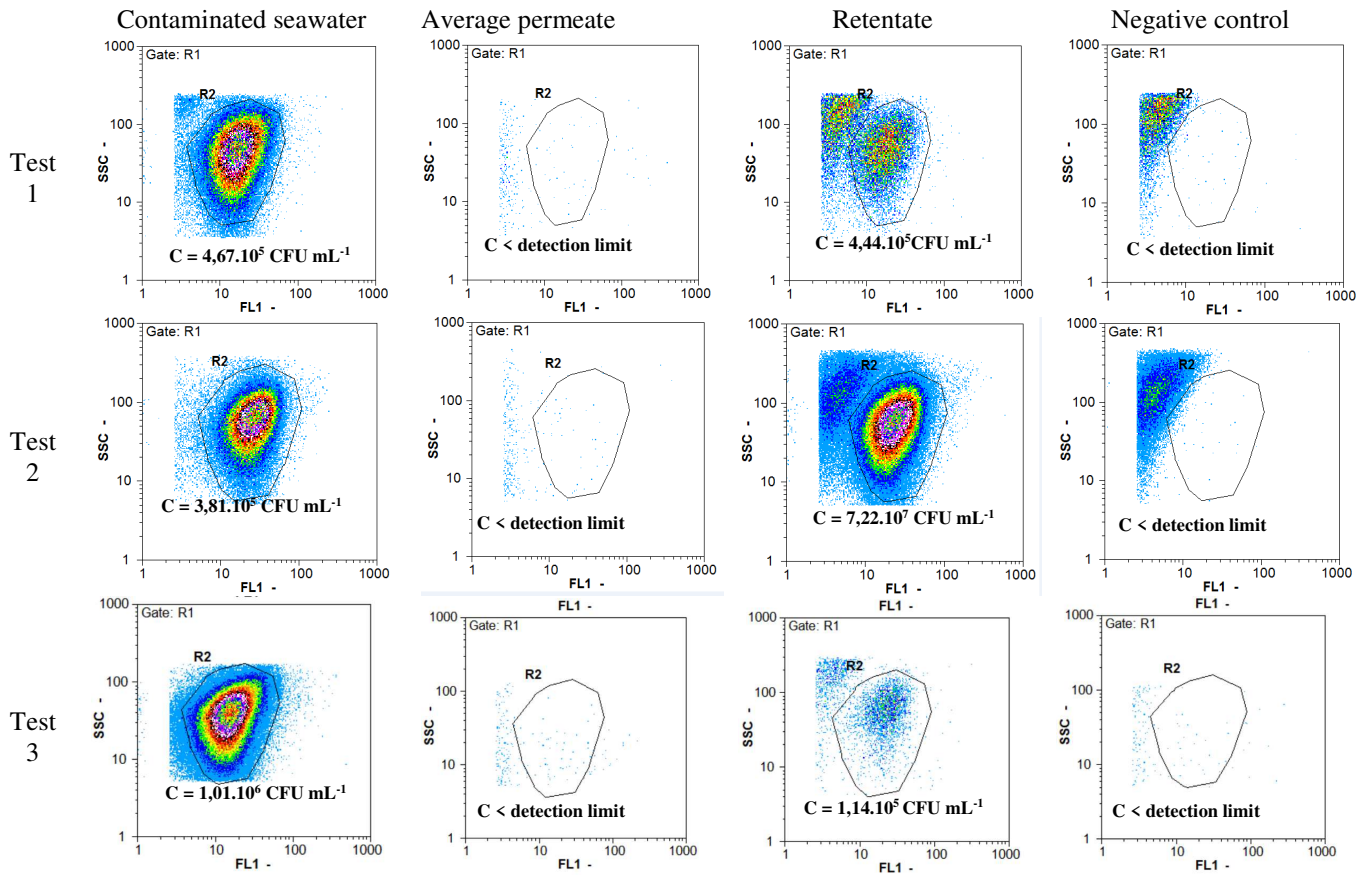


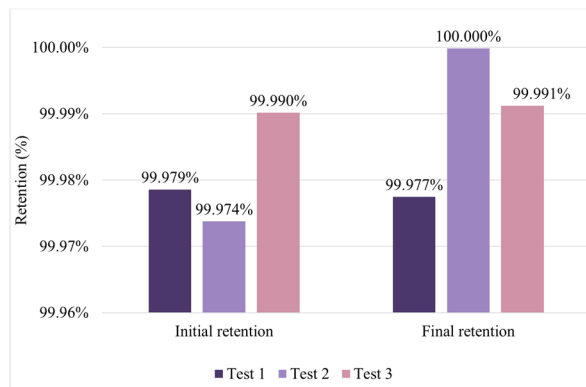
Figure 9: Treatment of *Vibrio aestuarianus* – Flow cytometry analysis

514
515

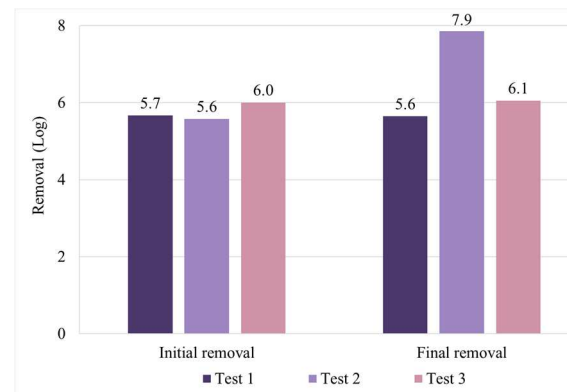
516 The initial and final retentions and removal rate of the 3 experiments are given in Figure 10.
517 These values were calculated with the flow cytometry detection limit (i.e. 100 CFU mL^{-1}) and
518 therefore underestimated .

519
520
521
522
523
524
525
526

a.



b.



527 *Figure 10: Treatment of Vibrio aestuarianus - a. Initial and final retention et b. Initial*
528 *and final removal – Flow cytometry analysis*
529

530 The results obtained by flow cytometry are in agreement with those obtained by the method of
531 inoculation on a Petri dish. In conclusion for *V. aestuarianus* bacteria, the three specific
532 experiments carried out with this bacterium highlight the retention of this microorganism,
533 allowing concentration levels in the permeate to be reached below the detection thresholds of
534 the different methods used. Indeed, the detection threshold of the analysis by flow cytometry
535 was 100 CFU mL⁻¹, which decreased to 20 CFU mL⁻¹ in Petri dish by direct inoculation of
536 50 µL, versus 2CFU L⁻¹ only for the method of depositing a 0.2 µm filter which had filtering
537 several liters of permeate. If *in vivo* tests could not be carried out to validate the efficacy of the
538 treatment, a study by Travers *et al.* (2017) estimated the minimum infective dose required to
539 reliably induce infection in adult oysters after a 24 h immersion period in contaminated
540 seawater, was estimated at 4 × 10⁴ CFU mL⁻¹, i.e. 400 times more than the highest detection
541 limit in this study. With these results, it is possible to conclude on the effectiveness of the
542 process to protect oyster farms from this pathogenic agent.

543

544 **III.2. Disinfection of water entering the hatchery / nursery on a semi-industrial scale**

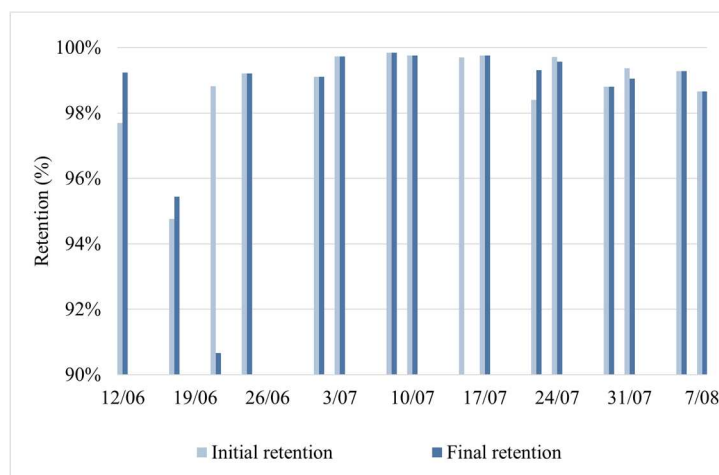
545 The objective is to validate the use of ultrafiltration for the disinfection of water entering the
546 hatchery / nursery on a semi-industrial scale and for real conditions in feed water. The hydraulic
547 performance of the process is therefore monitored over several months and the retention of the
548 total flora and *Vibrio* bacteria naturally present in the water supplying the pilot are evaluated.
549 During the period of these tests, the pilot was confronted with different qualities of seawater
550 and even in the particular case of an algal bloom upstream of the experimental installations.

551

552 III.2.1. Bacteria removal rate

553 The retention performance of total bacteria is presented in Figure 11. The measurements were
554 carried out at the beginning (initial permeate) and at the end of the filtration cycle (final
555 permeate). Samples from June 12 and June 17 (2019) show less than 98% of the retention,
556 which is explained by the formation of a biofilm in the permeate lines. After cleaning the pilot
557 with chlorine at 4 ppm on June 24, the retention of the total flora is greater than 98 %, thus
558 reflecting the effectiveness of the membrane. The concentration of total flora in the permeates
559 is below the detection threshold of the analyze (20 CFU mL^{-1}), regardless of the bacterial
560 concentration of the feed. This detection limit, coupled with concentrations in the feed of around
561 10^4 CFU mL^{-1} , explains the low removal rate of only 99 %.

562

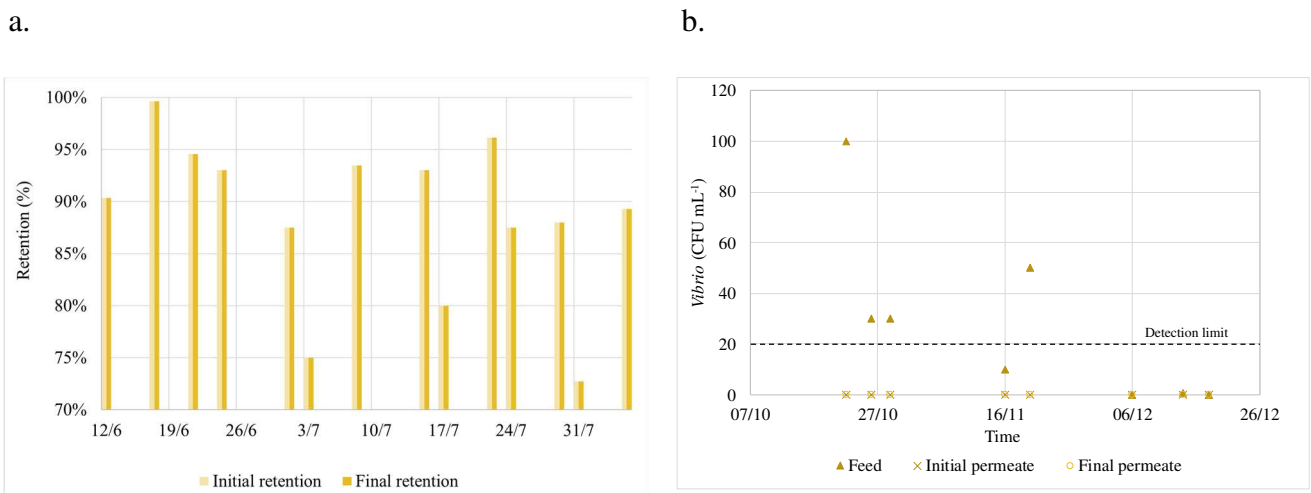


563

564
565

Figure 11: Total flora retention – Semi-industrial scale

566 During the two months of follow-up, no *Vibrio* bacteria was detected on the permeate samples
 567 (initial and final). The concentration fixed to calculate the retention Figure 12, 20 CFU mL⁻¹
 568 which is the detection limit justifies the low retention obtained. The initial concentrations of
 569 bacteria of the genus *Vibrio* remained low during the follow-up (200 CFU mL⁻¹ on average).
 570 Some peaks were observed in the feed water on 21 June with 5220 CFU mL⁻¹ and on 24 July
 571 with 520 CFU mL⁻¹. Despite these fluctuations in bacterial concentrations, the membrane
 572 process has enabled retention since the bacteria are not quantifiable in the permeates.
 573 Ultrafiltration ensures the retention of *Vibrio* bacteria regardless of the initial concentration, the
 574 permeability of the membrane and the physico-chemical parameters of the seawater. Similar
 575 results leading to the same conclusion were obtained in winter Figure 12b.



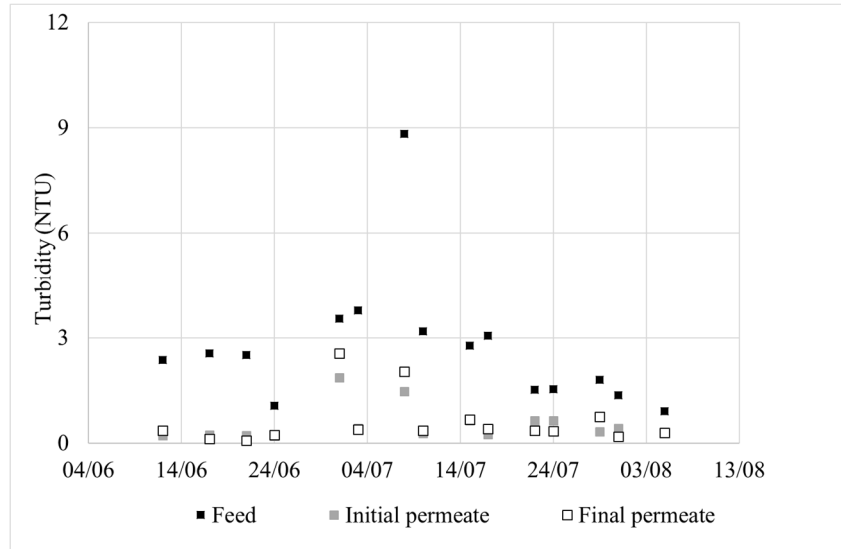
576 *Figure 12: a. Evolution of Vibrio retention (June - July 2019) and b. Evolution of Vibrio*
 577 *concentrations (October – December 2018)*
 578

579 III.2.2. Effect on the physico-chemical parameters

580 The physico-chemical parameters of the feed and initial and final permeates were followed for
 581 2 months. As expected, the pH, salinity, temperature and dissolved oxygen are not affected by
 582 the ultrafiltration treatment since the values are constant between the feed and permeates
 583 (Cordier et al., 2019b). Conversely, in Figure 13, a reduction in the turbidity is obtained with

584 the ultrafiltration process since it is mainly below 1 NTU in the permeates whatever the
585 turbidity of the feed (between 1 and 9 NTU).

586



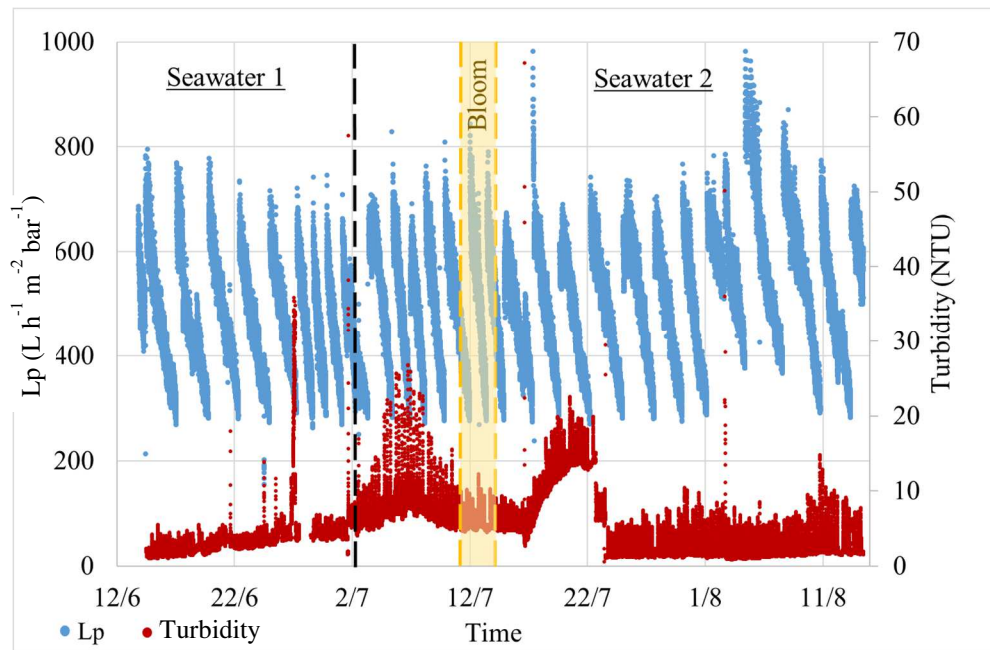
587

588 *Figure 13: Evolution of turbidity vs. time (June - August 2019)*

589

590 III.2.3. Hydrodynamic performances

591 The evolution of permeability and turbidity versus time is presented in Figure 14. The peaks of
592 turbidity recorded every 6 hours correspond to the backwashing of the sand filter placed
593 upstream of the ultrafiltration pilot plant (the peak of turbidity between 07/16 and 07/23 is not
594 representative of the water quality- probe position error). Whatever the water quality with a
595 turbidity from 3-7 NTU, the slopes relating to the membrane fouling are more significant for
596 high turbidity. The time between two chemical cleanings is therefore shorter: before the
597 increase of turbidity from 3-4 to 6-7 NTU, the time to pass from a permeability of 600 at 300 L
598 $\text{h}^{-1} \text{m}^{-2} \text{bar}^{-1}$ is on average 47.4 h against 24.1 h after the change in water quality.



599

600 *Figure 14: Evolution of permeability of turbidity vs. time (June – August 2019) – Semi-industrial scale*
 601 *pilot*

602

603 If an increase in turbidity has an impact on the fouling, this has no effect on the recovery of
 604 initial permeability obtained after a chemical cleaning (CEB). Indeed the average initial
 605 permeability is $750 \pm 50 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$ and this remained constant over the duration of the
 606 study and on the different qualities of water. Moreover, during this period an algal bloom was
 607 observed. Water treatment processes commonly used in the laboratory were not able to face the
 608 pollution and the consequence was a contamination of the oyster lines maintained in the
 609 experimental hatchery/nursery. On the contrary, ultrafiltration process showed its efficiency
 610 both in terms of hydraulic performances, with a stability of the permeability and retention of
 611 pollution (bacteria, turbidity, oyster predators) with the production of a clear water free of
 612 parasites (Cordier, 2019a)

613

614 **IV. Conclusions**

615 Performances of the ultrafiltration process were evaluated for biosecuring farms. The first part
616 was devoted to the validation of the process for the elimination of pathogenic organisms from
617 the seawater flow used in oyster hatchery/nursery. Laboratory-scale trials were used to meet
618 site security requirements, and focused on the two main pathogens affecting the French oyster
619 production: the OsHV-1 virus and the bacteria *Vibrio aestuarianus*. It emerges from this work,
620 that the retention of OsHV-1 was always greater than 98 %, but did not reach 100%. Meanwhile,
621 the quantity of viral DNA found for the permeate condition did not generate mortality using
622 bathing neither on spat nor larvae, the latter being very sensitive to this pathogenic agent. For
623 the bacterium *Vibrio aestuarianus*, the retention tests of the bacterium reduced by 5 to 7 on a
624 log scale for the permeate condition reaching the limits of detection regardless of the analytical
625 techniques (seeding on the medium of specific culture of permeate samples, permeate filtration
626 and culture of the filter on specific medium and flow cytometry). The permeate filtration tests
627 and re-culture of the filter highlight the recovered concentrations resistant to 2 CFU L⁻¹. If these
628 *in vitro* analyzes do not allow to conclude on the absence of virulence of the permeate on
629 oysters, the tests carried out by Travers *et al.* (2017) found a concentration 400 times higher to
630 impact the adult oysters by bathing. Thus, protection of oyster farms at the larval, spat and adult
631 stages were obtained by the ultrafiltration process against two pathogens known worldwide for
632 their impact on the *Crassostrea gigas* oyster production. Tests on an industrial scale, which
633 were carried out naturally with lower but real concentrations of microorganisms, validate
634 ultrafiltration in real conditions as a treatment for incoming seawater from shellfish structures.
635 Bacterial abatement monitoring highlights retention with concentrations in the permeate below
636 the detection threshold for analysis of *Vibrio* bacteria, potentially pathogenic for shellfish. This
637 was confirmed over several months of analysis, different water qualities and bacterial loads
638 upstream of the membranes. Regarding hydraulic and retention performances, the process was
639 tested on two water qualities including during an algal bloom. The permeability remained

640 constant after the chemical cleanings, and the time between these cleaning procedures did not
641 drop below 12 h over the duration of the study, reflecting the resistance and the stability of the
642 process.

643

644 **ACKNOWLEDGEMENT**

645 The project leading to this publication has received funding from FEAMP (R FEA 4700 16FA
646 100 0001). We warmly thank Bruno Petton and the staff of the Ifremer station of Bouin
647 (PMMB) for technical support in the production of biological material and Mickael Mege for
648 virus extraction.

649

650

651

652

653

654

655 **Références**

- 656 Aboubaker, M.H., Sabrié, J., Huet, M., Koken, M., 2013. Establishment of stable GFP-tagged
657 *Vibrio aestuarianus* strains for the analysis of bacterial infection-dynamics in the Pacific
658 oyster, *Crassostrea gigas*. *Vet. Microbiol.* 164, 392–398.
659 <https://doi.org/10.1016/j.vetmic.2013.02.024>
- 660 Azéma, P., Lamy, J.-B., Boudry, P., Renault, T., Travers, M.-A., Dégremont, L., 2017. Genetic
661 parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific
662 oyster, *Crassostrea gigas*, at three different life stages. *Genet Sel Evol* 49, 23.
663 <https://doi.org/10.1186/s12711-017-0297-2>
- 664 Azéma, P., Travers, M.-A., Benabdelmouna, A., Dégremont, L., 2016. Single or dual
665 experimental infections with *Vibrio aestuarianus* and OsHV-1 in diploid and triploid
666 *Crassostrea gigas* at the spat, juvenile and adult stages. *Journal of Invertebrate Pathology*
667 139, 92–101. <https://doi.org/10.1016/j.jip.2016.08.002>
- 668 Azéma, P., Travers, M.-A., De Lorgeril, J., Tourbiez, D., Dégremont, L., 2015. Can selection
669 for resistance to OsHV-1 infection modify susceptibility to *Vibrio aestuarianus*
670 infection in *Crassostrea gigas*? First insights from experimental challenges using
671 primary and successive exposures. *Veterinary Research* 46, 139.
672 <https://doi.org/10.1186/s13567-015-0282-0>
- 673 Burnell, G., Allan, G., 2009. *New Technologies in Aquaculture: Improving Production*
674 *Efficiency, Quality and Environmental Management*. Elsevier.
- 675 Buestel, D., Ropert, M., Prou, J., Gouilletquer, P., 2009. History, status and future of oyster
676 culture in France. *Journal of Shellfish Research* 28, 813–820.
677 <https://doi.org/10.2983/035.028.0410>
- 678 Cochenec, N., Baud, J.-P., Pépin, J., Benabdelmouna, A., Soletchnik, P., Lupo, C., Garcia, C.,
679 Arzul, I., Boudry, P., Huvet, A., Pernet, F., Bachere, E., Bédier, E., Petton, B., Gaussem,
680 F., Stanisiere, J.-Y., Dégremont, L., 2011. Les surmortalités des naissains d’huîtres
681 creuses, *Crassostrea gigas* : acquis des recherches en 2010.
- 682 Cordier, C., 2019. L’ultrafiltration pour une meilleure maîtrise de la qualité de l’eau dans les
683 écloséries et nurseries conchylicoles - 2019AIXM0505. Aix-Marseille Université.
- 684 Cordier, C., L. Charpin, C. Stavrakakis, M. Papin, K. Guyomard, P. Sauvade, F. Coelho, P.
685 Moulin, 2019, Ultrafiltration: A solution to recycle the breeding waters in shellfish
686 production, *Aquaculture*. 504, 30–38. doi:10.1016/j.aquaculture.2019.01.045.
- 687 Cordier, C., Stavrakakis, C., Dupuy, B., Papin, M., Sauvade, P., Coelho, F., Moulin, P., 2019.
688 Ultrafiltration for environment safety in shellfish production: Removal of oyster
689 gametes in hatchery effluents. *Aquacultural Engineering* 84, 80–90.
690 <https://doi.org/10.1016/j.aquaeng.2018.12.008>
- 691 Cordier, C., Stavrakakis, C., Sauvade, P., Coelho, F., Moulin, P., 2018. Air Backwash
692 Efficiency on Organic Fouling of UF Membranes Applied to Shellfish Hatchery
693 Effluents. *Membranes* 8, 48. <https://doi.org/10.3390/membranes8030048>
- 694 Dégremont, L., 2013. Size and genotype affect resistance to mortality caused by OsHV-1 in
695 *Crassostrea gigas*. *Aquaculture* 416–417, 129–134.
696 <https://doi.org/10.1016/j.aquaculture.2013.09.011>
- 697 Dégremont, L., Ledu, C., Maurouard, E., Nourry, M., Benabdelmouna, A., 2016a. Effect of
698 ploidy on the mortality of *Crassostrea gigas* spat caused by OsHV-1 in France using
699 unselected and selected OsHV-1 resistant oysters. *Aquaculture Research* 47, 777–786.
700 <https://doi.org/10.1111/are.12536>
- 701 Dégremont, L., Morga, B., Trancart, S., Pépin, J., 2016b. Resistance to OsHV-1 Infection in
702 *Crassostrea gigas* Larvae. *Frontiers in Marine Science* 3.
703 <https://doi.org/10.3389/fmars.2016.00015>

704 Delacroix, S., Vogelsang, C., Tobiesen, A., Liltved, H., 2013. Disinfection by-products and
705 ecotoxicity of ballast water after oxidative treatment--results and experiences from
706 seven years of full-scale testing of ballast water management systems. *Mar. Pollut. Bull.*
707 73, 24–36. <https://doi.org/10.1016/j.marpolbul.2013.06.014>

708 Ford, S.E., Xu, Z., Debrosse, G., 2001. Use of particle filtration and UV irradiation to prevent
709 infection by *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in
710 hatchery-reared larval and juvenile oysters. *Aquaculture* 194, 37–49.
711 [https://doi.org/10.1016/S0044-8486\(00\)00507-X](https://doi.org/10.1016/S0044-8486(00)00507-X)

712 Greenlee, L.F., Lawler, D.F., Freeman, B.D., Marrot, B., Moulin, P., 2009. Reverse osmosis
713 desalination: Water sources, technology, and today's challenges. *Water Research* 43,
714 2317–2348. <https://doi.org/10.1016/j.watres.2009.03.010>

715 Gullian, M., Espinosa-Faller, F.J., Núñez, A., López-Barahona, N., 2012. Effect of turbidity on
716 the ultraviolet disinfection performance in recirculating aquaculture systems with low
717 water exchange. *Aquaculture Research* 43, 595–606. <https://doi.org/10.1111/j.1365-2109.2011.02866.x>

719 Helm, M., 2004. The hatchery culture of bivalves: a practical manual. FAO.

720 Karakulski, K., Gryta, M., Morawski, A., 2002. Membrane processes used for potable water
721 quality improvement. *Desalination* 145, 315–319. [https://doi.org/10.1016/S0011-9164\(02\)00429-0](https://doi.org/10.1016/S0011-9164(02)00429-0)

723 Kornmueller, A., 2007. Review of fundamentals and specific aspects of oxidation technologies
724 in marine waters. *Water Sci Technol* 55, 1–6. <https://doi.org/10.2166/wst.2007.379>

725 Lazarova, V., Savoye, P., Janex, M.L., Blatchley, E.R., Pommepuy, M., 1999. Advanced
726 wastewater disinfection technologies: State of the art and perspectives. *Water Science
727 and Technology* 40, 203–213. [https://doi.org/10.1016/S0273-1223\(99\)00502-8](https://doi.org/10.1016/S0273-1223(99)00502-8)

728 Lekang, O.-I., 2013. *Aquaculture engineering, Second Edition.* ed. Wiley-Blackwell,
729 Chichester, West Sussex, UK.

730 Martínez, L.F., Mahamud, M.M., Lavín, A.G., Bueno, J.L., 2013. The regrowth of
731 phytoplankton cultures after UV disinfection. *Mar. Pollut. Bull.* 67, 152–157.
732 <https://doi.org/10.1016/j.marpolbul.2012.11.019>

733 Moretti, A., Fernandez-Criado, M.P., Cittolin, G., Guidastrri, R., 1999. Manual on hatchery
734 production of seabass and gilthead seabream: Volume 1.

735 Morga, B., Picot, S., Faury, N., Renault, T., Dégremont, L., Lupo, C., Travers, M.-A., 2020.
736 OsHV-1 infection in Pacific oyster, *Crassostrea gigas*: does first barrier passage shunt
737 matter in disease reproducibility? *Journal of Invertebrate (under press)*.

738 Petton, B., Boudry, P., Alunno-Bruscia, M., Pernet, F., 2015. Factors influencing disease-
739 induced mortality of Pacific oysters *Crassostrea gigas*. *Aquacult. Environ. Interact.* 6,
740 205–222. <https://doi.org/10.3354/aei00125>

741 Powell, A., Scolding, J.S.W., 2016. Direct application of ozone in aquaculture systems *Reviews
742 in Aquaculture*, 424–438. <https://doi.org/10.1111/raq.12169>

743 Qualls, R.G., Johnson, J.D., 1983. Bioassay and dose measurement in UV disinfection. *Appl
744 Environ Microbiol* 45, 872–877.

745 Renault, T., 2011. A review of mortality outbreaks in the Pacific oyster, *Crassostrea gigas*,
746 reported since 2008 in various European Union Member States and the related
747 implementation of Council Directive 2006/88/EC 2006/88/EC 2, 2.

748 Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., Demarini, D.M., 2007. Occurrence,
749 genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in
750 drinking water: a review and roadmap for research. *Mutat. Res.* 636, 178–242.
751 <https://doi.org/10.1016/j.mrrev.2007.09.001>

752 Schikorski, D., Renault, T., Saulnier, D., Faury, N., Moreau, P., Pépin, J.-F., 2011.
753 Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus

754 1: demonstration of oyster spat susceptibility. *Vet Res* 42, 27.
755 <https://doi.org/10.1186/1297-9716-42-27>

756 Sharrer, M.J., Tal, Y., Ferrier, D., Hankins, J.A., Summerfelt, S.T., 2007. Membrane biological
757 reactor treatment of a saline backwash flow from a recirculating aquaculture system.
758 *Aquacultural Engineering* 36, 159–176. <https://doi.org/10.1016/j.aquaeng.2006.10.003>

759 Solomieu, V., Renault, T., Travers, M.-A., 2015. Mass mortality in bivalves and the intricate
760 case of the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* 131, 2–10.
761 <https://doi.org/10.1016/j.jip.2015.07.011>

762 Souissi, Y., Bouchonnet, S., Bourcier, S., Kusk, K.O., Sablier, M., Andersen, H.R., 2013.
763 Identification and ecotoxicity of degradation products of chloroacetamide herbicides
764 from UV-treatment of water. *SCI TOTAL ENVIRON* 458, 527–534.
765 <https://doi.org/10.1016/j.scitotenv.2013.04.064>

766 Stavrakakis, C., Papin, M., Dupuy, B., Riou, K., Penisson, C., Nourry, M., Hatt, P.-J., Buchet,
767 V., Palvadeau, H., 2017. Désinfection de l'eau de mer – DESIMER Etude des sous-
768 produits de désinfection (Report (Contract report)). FRANCE.

769 Summerfelt, S.T., 2003. Ozonation and UV irradiation—an introduction and examples of
770 current applications. *Aquacultural Engineering* 28, 21–36.
771 [https://doi.org/10.1016/S0144-8609\(02\)00069-9](https://doi.org/10.1016/S0144-8609(02)00069-9)

772 Suquet, M., Kermoysan, G. de, Araya, R.G., Queau, I., Lebrun, L., Souchu, P.L., Mingant, C.,
773 2009. Anesthesia in Pacific oyster, *Crassostrea gigas*. *Aquat. Living Resour.* 22, 29–34.
774 <https://doi.org/10.1051/alr/2009006>

775 Torgersen, Y., Hastein, T., 1995. Disinfection in aquaculture *Revue scientifique et technique*
776 de l'office international des epizooties, 419–434. <https://doi.org/10.20506/rst.14.2.845>

777 Travers, M.-A., Boettcher Miller, K., Roque, A., Friedman, C.S., 2015. Bacterial diseases in
778 marine bivalves. *J. Invertebr. Pathol.* 131, 11–31.
779 <https://doi.org/10.1016/j.jip.2015.07.010>

780 Travers, M.-A., Tourbiez, D., Parizadeh, L., Haffner, P., Kozic-Djellouli, A., Aboubaker, M.,
781 Koken, M., Dégremont, L., Lupo, C., 2017. Several strains, one disease: experimental
782 investigation of *Vibrio aestuarianus* infection parameters in the Pacific oyster,
783 *Crassostrea gigas*. *Veterinary Research* 48, 32. [https://doi.org/10.1186/s13567-017-](https://doi.org/10.1186/s13567-017-0438-1)
784 0438-1

785 Utting, S. d., Helm, M., 1985. Improvement of sea water quality by physical and chemical pre-
786 treatment in a bivalve hatchery. *Aquaculture*.

787 Wallace, R., Waters, P., Rikard, F., 2008. *Oyster Hatchery Techniques*.

788 Wolf, P.H., Siverns, S., Monti, S., 2005. UF membranes for RO desalination pretreatment.
789 *Desalination, Desalination and the Environment* 182, 293–300.
790 <https://doi.org/10.1016/j.desal.2005.05.006>

791 Zhang, Q., Gui, J.-F., 2015. Virus genomes and virus-host interactions in aquaculture animals.
792 *Sci. China Life Sci.* 58, 156–169. <https://doi.org/10.1007/s11427-015-4802-y>

793

794