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1 **Evaluation of membrane filtration and UV irradiation to control bacterial loads in** 2 **recirculation aquaculture systems**

3

4 David Huyben^{a*}, David Bevan^a, Roselynn Stevenson^b, Hongde Zhou^c, and Richard Moccia^a

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6 ^a Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, N1G 2W1;

7 ^b Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada, N1G 2W1;

8 ^c School of Engineering, University of Guelph, Guelph, ON, Canada, N1G 2W1

9 * Corresponding author. Email: David Huyben, davidhuyben@gmail.com, +44-73983-58847.

10

11 **Abstract**

12

13 Ultraviolet (UV) irradiation is commonly used to control pathogen loads in recirculation aquaculture systems (RAS),
14 although these micro-organisms can be shielded by particles in the water, and some species tolerate very high UV
15 doses. The objective of this study was to evaluate membrane filtration (MF) as an alternative, or complimentary,
16 treatment to UV irradiation for pathogen control in RAS, as well as examine the operation and cost of each treatment.
17 In a pilot-scale RAS, both MF and UV were used to treat wastewater for 30 days and water samples were collected
18 biweekly and analysed for culturable bacteria, suspended solids, UV transmittance and other parameters. Bacterial
19 control efficiencies were similar between both MF and UV treatments, which removed 99% of total bacteria and 98%
20 of heterotrophic bacteria, respectively. Surface fouling was negligible for the UV while MF required biweekly
21 cleaning to maintain operation. However, MF had the additional benefit of removing 96% of suspended solids, which
22 resulted in increased UV transmittance. Capital and operating costs of MF were similar to UV, but only when MF
23 treated a fraction of the wastewater compared with UV. We conclude that MF represents a potential complimentary
24 technology to enhance UV irradiation, especially to minimise pathogens in RAS that are shielded by particles or
25 tolerate UV.

26

27 **Keywords**

28 Bacterial removal - Fine solids removal – RAS biosecurity - Wastewater treatment - UV tolerant bacteria

29

30 **Shortened title:**

31 Membrane filtration of bacteria in RAS

32

33 **1. Introduction**

34

35 Recirculation aquaculture systems (RAS) use various combinations of water treatment technologies to remove
36 pathogens and other wastes derived from fish excretions and uneaten feed, in order to reuse and supply high quality
37 water to farmed fish (Piedrahita, 2003). Recirculation of waste materials and pathogens can impart added stress on
38 fish with associated morbidity, and can increase the prevalence of infection and clinical disease (Conte, 1992;
39 Wedemeyer, 1996). Micro-screen drum filters are typically used in RAS to remove wastes greater than 50 microns,
40 but smaller particles and pathogens can often bypass these micro-screens (Patterson et al, 1999). Ozone is often used
41 in RAS to deconstruct fine particles and pathogens, but using ozone can present a health risk to fish as well as humans
42 because it is toxic at low levels (Sharrer et al., 2005; Wedemeyer, 1996). UV irradiation is typically used in RAS
43 either alone, or in combination with ozone, to inactivate pathogens, but pathogens can sometimes tolerate this
44 treatment when “fouling” or high turbidity occurs, which shields pathogens from the UV exposure (Lazarova et al.,
45 1999; Liltved and Cripps, 1999; Wedemeyer, 1996). In addition, some pathogens can tolerate very high doses of UV,
46 and these proliferate within the RAS causing increased pathogen loads for the fish (Wedemeyer, 1996). For example,
47 *Flavobacterium psychrophilum* has been found to require four-fold higher UV doses than the recommended dose of
48 30 mJ/cm² (Sharrer et al., 2005) to achieve 5-log reductions of this pathogen (Hedrick et al., 2000). This bacterial
49 pathogen has been found in several aquaculture facilities of rainbow trout and in some cases can cause mortalities up
50 to 90% (Nilsen et al., 2011). Lastly, UV has been found to inactivate most, but not all bacteria in RAS, and this can
51 lead to the selection and proliferation of opportunistic pathogens that destabilizes the microbial community
52 (Attramadal et al., 2012). Therefore, ineffective control of pathogens using UV irradiation poses a potential risk to
53 fish health and the biocontrol aspects of RAS.

54 Membrane filtration (MF) is a process technology that physically separates solids from fluid using semi-
55 permeable membranes that can be classified based on pore size: either as microfiltration, ultrafiltration, nanofiltration
56 or reverse osmosis (Madaeni, 1999; Peters, 2010). Ultrafiltration membranes have pore sizes between 0.005 to 0.02
57 μm (Madaeni, 1999; Peters, 2010; Zhou and Smith, 2002) that allow dissolved ions and water to diffuse, while
58 retaining suspended particles, protozoa, bacteria, viruses and other waste components larger than the applied pore size
59 (Guo et al., 2009). Retained wastewater is continually or periodically drained from the MF system, but particles can
60 clog and adsorb onto, or within the membrane's pore structures that results in reduced filtration rates and higher
61 transmembrane pressures (TMP) (Le-Clech et al., 2006; Madaeni, 1999). Continual fouling and increases in TMP can
62 damage membranes. However, cleaning strategies, such as air-scouring and chemical washing can reduce fouling rates
63 and maintain filtration efficiency, which is essential for long-term operation (Le-Clech et al., 2006; Madaeni, 1999;
64 Zhou and Smith, 2002). In the past, MF technologies were considered costly, but advances in MF manufacturing
65 efficiency and its widespread use in other sectors have increased its affordability and potential application in the
66 aquaculture industry (Gomez et al., 2007). A study using a dead-end MF module with a pore size of 0.05 μm removed
67 94% of suspended solids with a 76% reduction in biochemical oxygen demand from aquaculture wastewater (Viadero
68 and Noblet, 2002). However, MF has only been evaluated for solids and bacterial removal on a lab-scale, thus larger
69 scale studies in RAS are needed in comparison to established technologies, e.g. UV.

70 Therefore, due to its various properties, MF technology may present a viable alternative or complimentary
71 treatment to UV for the control of fish pathogens in RAS. The objective of this study was to compare MF and UV in
72 terms of the removal efficiency of culturable bacteria, additional effects on water quality, fouling resistance and
73 capital/operating costs in a pilot-scale RAS.

74

75 **2. Materials and methods**

76

77 **2.1 Recirculation Aquaculture System**

78 The study was conducted at the Alma Aquaculture Research Station (University of Guelph, Elora, ON, Canada) using
79 a warm-water (i.e. 20-23 °C) RAS that held Nile tilapia (*Oreochromis niloticus*) broodstock. The 5,600 L system was
80 composed of twelve circular fibreglass tanks (340 L) that contained 271 fish in total with a mean weight of 1.3 ± 0.2

81 kg (\pm standard deviation). The fish were fed a commercial diet by automatic belt feeders. The study was carried out
82 in accordance with the criteria set out by the Canadian Council of Animal Care (CCAC, 2005).

83 In the RAS, wastewater from fish tanks (Figure 1) was treated using a combination of a 60 μ m rotating micro-
84 screen drum filter, foam fractionator, fluidized micro-bead biofilter, CO₂ gas stripper and compressed oxygen injection
85 system (PRAqua Technologies Ltd., BC, Canada). After oxygenation, the wastewater was disinfected by a 22 L
86 closed-channel UV reactor (Trojan UV Logic™ midflow model 02AM20, Trojan Technologies Inc., ON, Canada) at
87 a rate of 193.5 ± 4.7 L/min before recirculating to the fish tanks. The low-pressure UV reactor was capable of
88 producing a maximum UV dose of 60 mJ/cm² with two lamps at a wavelength of 254 nm and a maximum flow rate
89 of 227 L/min. The RAS recycled 98-99 % of the total flow and water lost through evaporation or drum filter cleaning
90 cycles was replaced with high quality well water (8.5°C).

91

92 **2.2 Membrane Filtration System**

93 The MF system was composed of a membrane element (LSU-1515, Toray Membrane Inc., CA, USA) submerged in
94 a vertical tank of water (1.8 m height x 0.2 m diameter), termed the “membrane tank” with an overflow port at the top
95 (Figure 2). The membrane filter was capable of withstanding a maximum TMP of 100 kPa and maximum flow rate of
96 33 L/min. For more info on the MF system, see Table 1. Before UV treatment, wastewater was diverted into the MF
97 system in order to supply both MF and UV systems with exactly the same source and quality of wastewater. After
98 influent wastewater entered the MF system, two streams were produced; water that diffused through the membranes,
99 termed “permeate”, and wastewater that was retained outside the membranes and drained, termed “retentate”. Influent
100 wastewater, permeate and retentate flow rates were adjusted every two or three days to approximately 16, 15 and 1
101 L/min respectively. Retentate was continuously discarded and the permeate was recirculated to the collection sump
102 (Figure 1).

103 Three cleaning strategies were applied to the MF system at different intervals to control membrane fouling:
104 continuous air-scouring, membrane relaxation cycles and maintenance cleaning. For air-scouring, air was generated
105 at a rate of 25 L/min connected to an air stone at the bottom of the membrane tank (Figure 2). For relaxation cycles, a
106 repeat cycle timer turned off the permeate pump for one minute every 30 minutes to enhance air-scouring. For
107 maintenance cleaning, the MF was taken off-line, soaked in a solution of 300 mg/L (0.03 %) sodium hypochlorite for

108 30 minutes, rinsed with well water for 90 minutes and then re-installed in the RAS after no free chlorine (Hach Method
109 4500-Cl) was detected in the permeate stream (Toray Membrane Inc., CA, USA). The TMP was measured and
110 recorded every minute using a pressure transducer connected to a data logger (Lascar Electronics Inc., PA, USA),
111 which was used to calculate membrane fouling resistance (see section 2.4).

112

113 **2.3 Water Sampling and Analyses**

114 The MF system processed wastewater from the RAS, in parallel with the UV system, for 30 days. Water samples were
115 collected, in triplicate, from three sampling locations within the RAS: 1. before MF/UV treatment (influent), 2. after
116 MF treatment (effluent) and, 3. after UV treatment (effluent; Figure 1 and 2). Water samples were collected every two
117 or three days, and each sample replicate was taken five minutes apart. For bacterial analyses, separate water samples
118 were collected in triplicate into sterile bottles, from the three sampling ports within the RAS and then placed on ice
119 for later analysis.

120 Water samples were measured for total bacteria counts using the NEO-GRID/ISO-GRID membrane filtration
121 system (Neogen Corp., MI, USA) as described in the Official Methods of Analysis 986.32 (AOAC, 2007). An aliquot
122 of each sample was vacuum-filtered, in duplicate, on an iso-grid, placed on tryptic soy agar (Sigma-Aldrich Co., MO,
123 USA) and incubated for three days at 18 °C. After incubation, colonies were counted, converted to the corresponding
124 Most Probable Number (MPN) and then multiplied by the dilution factor to obtain colony forming units (CFU) /mL
125 of total bacteria (AOAC, 2007). Heterotrophic bacteria were measured at the Agriculture and Food Laboratory
126 (Guelph, ON, Canada) according to Standard Method 9215 (APHA, 1998).

127 For chemical water quality, influent and effluent samples were measured for dissolved oxygen, temperature and
128 pH using handheld probes (Oxyguard A/S, Farum, Denmark). The UV transmittance ($10^{-(UV \text{ absorbance} / \text{cm}) \times 100}$)
129 was measured at a 1 cm path length and a wavelength of 254 nm using a spectrophotometer (Hewlett-Packard
130 Co., CA, USA). Turbidity and total dissolved solids were measured using a turbidimeter (HF Scientific, Fort Myers,
131 FL, USA) and a spectrophotometer (Hach, London, Canada). Total suspended solids were measured using Standard
132 Method 2560 (APHA, 1998).

133

134 **2.4 Data and Cost Analyses**

135 Removal efficiencies achieved by MF and UV treatments were calculated for each set of water samples (((influent -
136 effluent)/influent) x 100%). For bacterial analyses, percent removal was used to calculate Log₁₀ reductions (-log₁₀(1 -
137 (% removal/100)) (Metcalf and Eddy, 2003). UV doses were calculated based on the UV intensity and water
138 characteristics ((UV intensity)*(exposure time)*(transmittance factor), mJ/cm²) (Sharrer et al., 2005).

139 Fouling resistance experienced by the MF system was calculated based on Darcy's equation (Belfort et al., 1994):

$$140 J = \frac{\Delta \text{TMP}}{\eta(R_m + R_f)}$$

141 where J is permeate flux (i.e. 35.78 L/m²/hr), ΔTMP is change in transmembrane pressure (kPa), η is water viscosity
142 at 22 °C (i.e. 0.02 kPa·min), R_m is membrane resistance against clean water during the first hour (i.e. 3.34x10⁵ /m)
143 and R_f is fouling resistance (/m). Fouling rates were calculated using fouling resistance values and time (t) between
144 each maintenance cleaning (i.e. (R_{f,t2} - R_{f,t1})/(t₂ - t₁), /m/day (Fan and Zhou, 2007).

145 A Shapiro-Wilk test was performed on data from each water quality parameter to determine data normality (Field
146 et al., 2012). To test for a difference in removal efficiencies between MF and UV treatments, two-sample Student's t-
147 test was performed on data sets that were normally distributed and a two-sample Wilcoxon signed-rank test was
148 performed on data sets that were not (Field et al., 2012). All statistical analyses were completed using R[®] version
149 2.11.1 software (R Core Development Team, 2011) and p-values below 0.05 were considered significant.

150 The capital and operating costs of the small pilot-scale MF and UV systems were calculated based on quotes
151 (USD\$) from commercial manufacturers (i.e. Toray Membrane and Trojan Technologies). The cost of pumps and
152 piping was calculated based on an assumption of 1.5 times the cost of the MF module based on the extra need for a
153 reversible permeate pump, air pump and membrane tank while the cost was 0.5 times the cost of UV, as according to
154 Cheryan (1998) and Viadero and Noblet (2002). The electricity costs (\$0.12/kWh; USA average 2011) for MF were
155 based on consumption from a 0.24 kW permeate pump and 0.17 kW air pump while the UV system used a 0.35 kW
156 permeate pump and 0.30 kW UV reactor. Tax and labour were excluded since it varies based on location, water quality
157 and automation of the treatments.

158

159 **3. Results**

160

161 In the 30-day study, both MF and UV treatments were very effective at achieving high removal efficiencies of
162 both total and heterotrophic bacteria (Table 2). No significant differences existed in total (Wilcoxon test; $W = 50$, $n =$
163 12 , $p = 0.085$) or heterotrophic ($W = 12$, $n = 5$, $p = 1.0$) bacteria between MF and UV treatments. However, two counts
164 of total bacteria (i.e. 1300 and 1500 CFU/mL) from MF effluent collected on day four and seven were not included in
165 the analysis because they were four-fold higher than influent levels, which indicated bacterial contamination inside
166 the membrane filter. Thereafter, the membrane filter was disinfected (maintenance cleaning) every two or three days
167 afterwards to reduce contamination.

168 Mean UV intensity and transmittance were 17.4 ± 0.2 mW/cm² and 92.0 ± 0.5 %, which produced a mean UV
169 dose of 110.0 ± 2.0 mJ/cm². The UV system did not require any cleaning because UV intensity did not drop below
170 the recommended minimum of 7.5 mW/cm².

171 Continuous air-scouring, membrane relaxation cycles and maintenance cleanings were effective at reducing
172 fouling of the MF system (Figure 2). After the first maintenance cleaning, the MF system achieved a mean fouling
173 rate of $7.04 \pm 1.19 \times 10^4$ /m/day, or a change in TMP of 0.66 ± 0.11 kPa/day based on nine filtration cycles. On day
174 0, the MF system had an initial TMP of 3.1 kPa (membrane resistance) and after 30 days of operation it had a final
175 TMP of 22.9 kPa. In addition to bacteria, MF achieved significant removal efficiencies of turbidity and total suspended
176 solids and had a significant effect on UV transmittance, pH and dissolved oxygen (Table 3).

177 The cost comparison showed that one MF element had lower capital and operating costs than UV, but less
178 wastewater would be treated (Table 4). In order to filter the same flow as UV, six MF elements would be required,
179 consequently increasing the capital and operating costs of the MF system to 3.8 times that of comparable UV system.

180

181 **4. Discussion**

182

183 This study demonstrated for the first time that MF can achieve similar bacterial removal efficiencies compared with
184 UV treatment in RAS, at least at a small, pilot-scale level. Recent studies have evaluated membrane bioreactors (MBR)
185 for bacteria retention for nitrogen removal at low flow rates in RAS (Holan et al., 2014; Sharrer et al., 2007; Wold et
186 al., 2014), although this is the first study to investigate bacterial removal at high flow rates in RAS. The MF system
187 achieved approximately 2 log reductions of total bacteria, which was similar to previous MF studies (Guo et al., 2009;

188 Nakatsuka et al., 1996). In comparison, the UV system achieved 2 log reductions while a study by Sharrer et al. (2005)
189 only achieved 0.5 and 0.7 log reductions of heterotrophic bacteria from a commercial RAS at UV doses of 78 and 150
190 mJ/cm². However, MF and UV systems in Sharrer et al. (2005) were challenged with approximately 10-fold and 2.5-
191 fold higher levels of bacteria and suspended solids compared with our study. High levels of suspended solids are
192 known to block UV transmittance and reduce UV disinfection efficiency (Gomez et al., 2007; Lazarova et al., 1999;
193 Wedemeyer, 1996). For example, Gomez et al. (2007) achieved higher pathogen removal efficiencies using MF
194 compared with UV when treating municipal wastewater with UV transmittance levels between 37-76%. In comparison
195 to our study, influent UV transmittance levels were higher (i.e. 89-95%), which suggested that the UV system was
196 able to achieve maximum bacterial removal and match the efficiency of the MF system. The MF treatment may have
197 been able to achieve higher bacterial removal efficiencies than UV if challenged with higher concentrations of
198 suspended solids and bacteria, but more research is required.

199 Since UV disinfection efficiency is reduced by suspended solids, using MF as a small side-stream treatment or
200 in combination with UV may be a better alternative. In this study, MF achieved high removal efficiency of suspended
201 solids, which in turn improved UV transmittance (Table 3). Thus, if MF were used as a pre-treatment in RAS with
202 more concentrated wastewater, the MF could remove particles that shield pathogens from UV and improve overall
203 UV disinfection efficiency (Gomez et al., 2007; Liltved and Cripps, 1999; Wedemeyer, 1996). In addition, the MF
204 system removed fine solids that bypassed micro-screen filtration that would have degraded in the rearing system and
205 resulted in the secondary production of ammonia and pathogenic bacteria that can negatively impact fish health
206 (Patterson et al., 1999; Piedrahita et al., 2003). Alternatively, MF could replace UV entirely and only treat part of the
207 wastewater, similar to a foam fractionator, to reduce bacteria and solids in RAS while avoiding negative effects of
208 UV. UV has been found to inactivate most bacteria and stimulate the rapid proliferation of opportunistic pathogens
209 (r-selection) in their place that can destabilise the microbial community (Attramadal et al., 2012; Wold et al., 2014).
210 The dominance of non-opportunistic bacteria (k-strategists) is proposed to inhibit proliferation of opportunistic
211 bacteria in RAS, at least for marine fish larvae (Skjermo et al., 1997). Therefore, constant removal of bacteria and
212 solids using MF would remove the substrate for bacteria and possibly prevent sporadic proliferation of opportunistic
213 pathogens in RAS. MF may be an alternative to the questionable long-term use of UV, especially since disease control
214 is still a major challenge in RAS that use UV.

215 A low rate of membrane fouling found in the present study indicates that MF has potential for long-term
216 operation in RAS as long as biweekly cleanings are performed. The highest TMP value achieved by the MF system
217 over the 30-day study (i.e. 25.2 kPa) did not reach the maximum value of 70 kPa, which requires recovery cleaning in
218 order to reduce TMP for continued operation. The membrane fouling rate may have been further reduced by applying
219 more cleaning strategies, such as permeate backwashing, while the three strategies employed in the present study were
220 sufficient. However, the MF system treated a low concentration of suspended solids, as mentioned previously, and
221 additional studies are needed to evaluate this technology in larger-scale RAS. In addition, a study that used a MBR to
222 remove nitrogen from a RAS of cod larvae (*Gadus morhua*) found that feeding dry feed resulted in higher membrane
223 fouling than feeding live feed (Holan et al., 2014). In comparison with UV, no maintenance or recovery cleaning
224 strategies were required, but again this may be due to low influent levels of suspended solids. Fouling is a larger
225 challenge for long-term operation of MF compared to UV, but further advances in membrane resistance, cleaning
226 strategies and design could reduce fouling rates and increase the long-term potential of MF in RAS.

227 New wastewater treatment technologies need to be affordable in order for their successful transition and use in
228 the aquaculture industry. Increased affordability was reflected in the present study as the cost of the single MF system
229 was cheaper and treated twice the flow rate of a similar MF system in RAS reported previously (Viadero and Noblet,
230 2002). The cost comparison showed that the advantage of MF over UV is the low capital cost of the MF elements and
231 their long lifespan, but the need for more pumps and frequent cleaning are disadvantages for MF. The capital cost of
232 the MF system with one element was lower than the UV system (Table 4). However, a MF system composed of six
233 elements are needed to treat the same flow rate as UV (i.e. 200 L/min) and this larger MF system would result in
234 greater than 1.5x the capital cost and 3.5x the operating cost. The higher cost of MF reduces its potential to completely
235 replace UV and treat a high flow rate of wastewater in RAS, but MF may be affordable as a small side-stream
236 treatment.

237 This study demonstrated that MF can achieve equivalent bacterial removal efficiencies compared to UV
238 irradiation in RAS. Removal of suspended solids and low membrane fouling rates achieved by MF indicate an
239 additional advantage and potential for long-term operation in RAS while frequent cleaning and higher
240 capital/operating cost of a larger MF system are potential disadvantages. Results from this study indicate that MF may
241 be best used as a side-stream treatment complementary to UV in RAS in order to minimise UV-shielded and tolerant

242 pathogens that induce disease. However, large-scale evaluations of MF in RAS under various operating conditions are
243 required before MF can be confidently used as a pathogen control measure in commercial aquaculture systems.

244

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246

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252

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