Integrity of reverse osmosis membrane for removing bacteria: New insight into bacterial passage

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Abstract

Water quality and reliability during potable reuse can often depend on the performance of the reverse osmosis (RO) membrane treatment for the attenuation of microbial contaminants. This pilot-scale study aimed to assess bacterial passage through intact RO membrane element and O-ring seal using stable fluorescent microspheres as bacterial surrogates and fluorescent stained bacteria. The removal of bacterial surrogates by three low pressure RO membrane elements varied considerably from 3.1 to 5.0-log (99.92% to 99.999%). O-ring seal bonding at the interface between RO feed and permeate streams increased the removal of bacterial surrogates by 0.2 to 0.4-log and the removal of actual bacteria in reclaimed water by 0.5-log. The results also show that conductivity is not a suitable surrogate parameter to monitor bacterial removal by these RO membranes. Overall, this study identified that even intact O-ring seal can allow for some bacterial passage and O-ring seal can be a source of low RO performance for bacterial removal. This study suggested the potential that the improvement of O-ring sealing performance can increase bacterial removal by 0.5-log.

Keywords: bacterial removal; fluorescent particle; membrane integrity; reverse osmosis; pressure vessel.

1 INTRODUCTION

Potable water reuse is a pragmatic, sustainable, and cost-effective strategy to augment water supply.[1-3] Direct potable reuse (DPR), which is the process of purifying reclaimed water to the same or above the current drinking water standard for distribution without an environmental buffer, has been increasingly considered for future potable reuse.[4, 5] The assurance of safety and reliability of water reuse for potable purposes is essential to its successful implementation.[6] In particular, the provision of public health protection from acute illnesses caused by microbial pathogens in wastewater is critical in DPR. Typical potable reuse schemes achieve attenuation of bacteria and other microbial contaminants of concern (e.g. virus and protozoa) through multiple treatment processes (often referred to as multiple barriers).[7, 8] Management of risks associated with bacterial pathogens is also a vital component in microbial water quality assurance of DPR.[9-11] For DPR, advanced water treatment facility has been suggested to achieve at least 9-log removal of total coliform bacteria.[12]

Among key treatment processes for potable water reuse, reverse osmosis (RO) is a arguably the most robust process capable of removing almost all constituents such as dissolved salts and trace organic chemicals. Although over 6-log removal of bacteria by RO treatment has been demonstrated in well controlled challenge test studies,[11] a removal value during direct integrity monitoring using tracer chemicals (e.g., Rhodamine WT dye) as surrogates can be as low as 2.5–4.0-log.[13] The accredited value for bacteria removal by RO through indirect integrity monitoring is even lower, from 1.5 to 2 log removal.[11, 14] This is because of the protocol for ensuring pathogen removal using rather conservative surrogate performance indicators: removal of total organic carbon (TOC) and/or electrical conductivity (EC).[9, 15] Fujioka et al.,[16] have recently

55 demonstrated the potential of continuously measuring bacterial removal by RO through real-time
56 counting of bacterial number in RO feed and permeate. Although the direct counting method was
57 expected to help ensuring bacterial removal considerably greater than conventional methods,
58 their study identified bacterial removal of lower than 3-log (99.9%).

59 Incomplete removal of bacteria by spiral-wound membrane elements of nanofiltration (NF) and
60 RO have been reported in the literature.[17-21] For example, high concentrations of bacteria in RO
61 permeate (total bacterial count of up to 1.2×10^3 counts/mL and heterotrophic plate count of 15
62 CFU/mL) have been reported at a full-scale plant.[19] Nevertheless, the cause of the presence of
63 bacteria after RO process has not been fully understood. RO membrane typically has free-
64 volume hole-diameter (or so-called pore size) less than 1 nm, whereas bacteria is considerably
65 larger in size (over 200 nm); thus, in theory the passage of bacteria through RO membrane sheet
66 is unlikely to occur. One potential location where bacterial passage could occur is the O-ring seal
67 that separates the feed and permeate during the assembly between two parts (RO membrane
68 elements and/or a pressure vessel).[13, 22] O-rings are located in the end-caps of a pressure vessel
69 and the interconnectors of RO membrane elements. Due to the need for manually replacing RO
70 membrane elements, the connections through O-ring seal can be a weak point where incomplete
71 sealing may occur.

72 Although several previous studies[23-26] have demonstrated membrane integrity breach by
73 intentionally damaging RO membrane components including O-rings, no previous study has
74 qualitatively evaluated the passage of bacteria through intact RO membrane elements. It is
75 difficult to control the concentration of biological substances (or makers) without bacterial
76 growth and death during an RO experiment. In contrast, stable bacterial surrogate substances
77 such as fluorescent (FL) microspheres are similar to bacteria in size but are not naturally present

in environment water;[27] thus, they are suitable for identifying the location of bacterial particle passage through RO. Understanding the location of bacterial passage through intact RO membrane element can make a breakthrough to the development of tight RO membrane system, ultimately leading to the improved safety of recycled water for potable reuse.

This study aimed to provide new insight to the passage of bacteria through intact RO membrane process. FL particle solutions and real reclaimed water were used in this evaluation. The contribution of intact O-ring seal to the passage of bacteria through intact RO membrane process was evaluated by bonding the O-ring seal using adhesive materials. The ultimate objective of this study was to provide an understanding on the location of bacterial passage through RO membrane for the improved removal of bacteria.

2 MATERIALS AND METHODS

2.1 RO membranes

All five membrane elements in this study were standard 4-inch spiral-wound and new **(Table 1)**. They include three low pressure RO (LPRO) membrane elements namely ESPA2, ESPA4 (Hydranautics/Nitto, CA, USA), and BW30 (Dow/Filmtec, MN, USA), denoted as Membrane A, B, and C, respectively. The forth element was a high pressure RO (HPRO) membrane commercially known as HYDRApro (Hydranautics/Nitto, CA, USA) dedicated for industrial uses under a high temperature condition. This is denoted as Membrane D. The fifth element was an RO membrane element permanently fitted to the pressure vessel (ESPA-FREE 3000L, Hydranautics/Nitto, CA, USA), in which RO feed and permeate streams are sealed without O-rings **(Fig. S1)**. This is denoted as Membrane E.

Table 1 – Specification of RO membrane elements provided by the manufacturers.

Name	Model	Manufacturer	Membrane area [m^2]	NaCl rejection [%]	Supply condition
A	ESPA2-LD-4040	Hydranautics	7.4	99.6	In preservatives
B	ESPA4-4040	Hydranautics	7.9	99.2	In preservatives
C	BW30-4040	Dow/Filmtec	7.2	99.5	Dry
D	HYDRApro-502-4040	Hydranautics	6.5	99.5	In preservatives
E	ESPA-FREE 3000L	Hydranautics	7.0	98.0	In preservatives

2.2 Pilot-scale RO system

A pilot-scale cross-flow RO filtration system **(Fig. S2)** was used in this study. The RO system consisted of one pressure vessel, a 65-L stainless steel reservoir, a high-pressure pump (25NED15Z, Nikuni Co., Ltd., Kawasaki, Japan), digital flow meters (FDM, Keyence Co., Osaka, Japan), digital pressure indicators (GPM, Keyence Co., Osaka, Japan), a pressure gauge, stainless steel pipes in the feed stream and PVC pipes and PTFE tubing in the permeate stream, and a titanium heat exchanging pipe connected to a chiller unit (CA-1116A, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). This study used an end-port (40E30N, Codeline/Pentair Water, Goa, India) or a side-port (R40B3001C, ROPV, Harbin, China) 4-inch fiberglass pressure vessel, respectively **(Fig. 1 and Fig. S3)**. The end-port pressure vessels were brand new, while the side-port pressure vessel had only been briefly used prior to this study. Poly-epoxy adhesive materials for bonding the O-ring seal between RO membrane element and pressure vessel were supplied with two-component liquids by Hydranautics/Nitto (Osaka, Japan).

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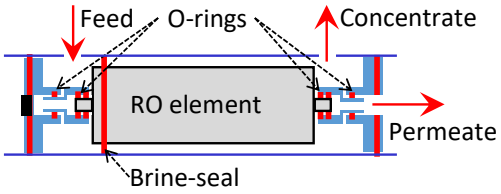
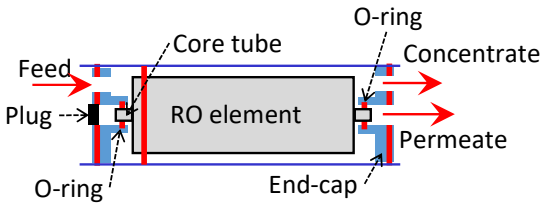
(a) Side-port



(b) End-port



115 **Fig. 1** – Schematic diagram of pressure vessel containing one RO element.

116 *2.3 Test solutions*

117 This study used Fluoresbrite® Yellow Green Carboxylate Microspheres 0.75 µm (Polysciences,
118 Inc., Warrington, PA, USA) as stable surrogate substances. According to the manufacture, the
119 FL particle stock solution was fluorescent polystyrene microspheres that have carboxylate
120 groups on their surfaces and their diameter was 0.75 µm (coefficient of variation in diameter =
121 3%). Tap water was collected in the laboratory at Nagasaki University Bunkyo Campus
122 (Nagasaki, Japan). Reclaimed water was obtained by applying ultrafiltration (UF) treatment to
123 secondary wastewater effluent from a wastewater treatment plant in Nagasaki, Japan. The UF-
124 treated wastewater was used for filtration experiments within three days after the sample
125 collection and preparation, and they were stored in the fridge until RO filtration experiments.

2.4 Analytical techniques

FL particle counting was conducted using a fluorescence microscope (Rapisco, Shibasaki Inc., Chichibu, Japan). RO feed water was diluted by 400 times using microfiltration (MF) membrane-treated pure water prior to analysis, whereas RO permeate did not undergo any dilution. Thereafter, 1 mL feed water and 50 mL permeate were filtered using a track-etched polycarbonate MF membrane with 0.2 μm pore size (Meric, Tokyo, Japan). The number of particles deposited on 40% of the filter surface area was counted and converted to particle count in 1 mL. When reclaimed water after UF treatment was used, bacterial counts were determined using the same protocol stated above. For fluorescent staining of DNA content in microbes, 4',6-diamidino-2-phenylindole (DAPI) dye (Thermo Fisher Scientific, Waltham, MA, USA) was used at 5 μg/mL. A real-time bacteriological counter (IMD-W^{TM}) from Azbil Corporation (Tokyo, Japan) was also used to continuously measure bacterial counts in RO permeate. The real-time bacteriological counter, which is based on two key technologies (particle size and auto-fluorescence detections), can count bacterial particles in real time without any chemical additions (e.g. fluorescent stains). Further details of the real-time instrument can be found elsewhere.[16] Conductivity of RO feed and permeate was analyzed using Orion Star™ A322 Conductivity meters (Thermo Fisher Scientific, Waltham, MA, USA).

2.5 Validation protocol

Each pilot-scale RO experiment was conducted in a closed-loop by recirculating RO concentrate and permeate into the feed reservoir **(Fig. S2)**. A pilot-scale cross-flow RO treatment was conducted using an approximately 50 L of tap water or UF-treated wastewater. When the removal of FL particles by various RO membrane elements was evaluated, the RO system was

operated at a constant permeate flux of 20 L/m^{2}h and a permeate recovery of 20% by adjusting transmembrane pressure (TMP). RO feed temperature was conditioned at 25 °C. It is noted that full-sale RO system is comprised of three stages with permeate recovery of 85%, recovery of the first stage is about 50%, and each pressure vessel typically holds 6 RO membrane elements in series. The pilot system in this study contained one RO membrane element. Thus, the permeate recovery of 20% in this study is to represent the first element in the first stage of a full scale RO plant. The RO system was first operated using tap water for over 60 min prior to the FL particle addition. Thereafter, a stock solution containing FL particles was spiked into the feed reservoir at the concentration of over 1×10^6 FL particles/mL. RO feed and permeate samples were periodically collected and FL particle concentrations were analyzed. When the removal of bacteria was evaluated, the RO system was first operated using RO-treated tap water for over 30 min. Thereafter, the RO-filtered tap water was replaced with a UF-treated wastewater. Bacterial counts in RO permeate were monitored online using a real-time bacteriological counter, while RO feedwater samples were collected at 10, 30 and 60 min for manual analysis using the real-time bacteriological counter.

3 RESULTS AND DISCUSSION

3.1 Bacterial passage through RO

3.1.1 Stability of fluorescent particle solution

The stability of FL particle (bacterial surrogate) solution in RO feed was evaluated to determine appropriate sample collection time for determining FL particle removal. The FL particle stock solution was dosed into the RO feed during the operation of RO system that contained Membrane A in an end-port pressure vessel. The concentration of FL particles in RO feed water

decreased gradually over time from approximately 1.1×10^6 to 6×10^5 counts/mL over 90 min of operation **(Fig. 2)**. It is noted that FL particles were well dispersed and did not aggregate in the feed reservoir **(Fig. S4)**. Thus, the decrease in FL particle count observed in this initial experiment can be attributed to the entrapment of FL particles within the complex feed channel structure of the RO membrane element **(Fig. S5)**. Corresponding to the decrease in FL particle concentration in the feed, FL particle concentrations in RO permeate also decreased gradually. Despite the reduction in FL particle concentration, the removal of FL particles remained constant at 4.9-log over the course of 90 min filtration experiment **(Fig. 2)**. Based on this preliminary data, the removal of FL particles during the following tests was calculated based on the average of FL particle removal determined at two sampling occasions (30 and 60 min).

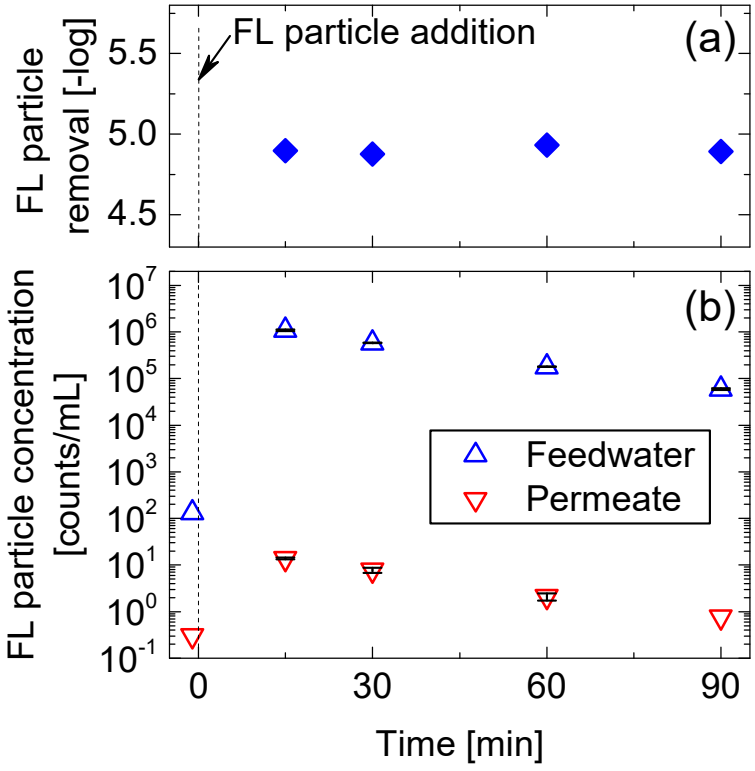


Fig. 2 – (a) Removal and (b) concentrations of FL particles during RO treatment of tap water containing FL particles using Membrane A. The symbols and error bars for FL particle concentrations are the average and standard deviation of two replicate samples.

3.1.2 Variation among RO membrane elements

The variation in FL particle removal among four RO membranes was evaluated. End-port pressure vessels were used for all membrane elements, whereas side-port pressure vessel was also used for Membrane A. Overall, a wide range of FL particle removal (between 3.1 and 7.1-log) was observed **(Fig. 3)**. Results in **Fig. 3** are consistent with the literature,[11] in which up to 6-log removal of bacteria has been reported in well controlled challenge test studies. This study examines low pressure RO membranes that have been typically used for water reuse applications. These membranes only have a moderate NaCl rejection **(Table 1)** and bacterial passage through pinhole-like defects within the membrane leaf is a possibility. In addition, any defects in the brine seal and membrane leaflet gluing may also contribute toward bacterial passage. The difference in membrane types (in terms of nominal NaCl rejection) and manufacturers can also explain for the range of FL particle removal observed in **Fig. 3**.

With respect to Membrane A, the type of pressure vessel as side- and end-port resulted in two different bacterial removal efficiencies of 5.0 and 4.7-log, respectively. Among the four RO membrane elements investigated along with an end-port pressure vessel, Membranes A and B showed similar removal of FL particles at 4.7 and 5.0-log, respectively. Membranes C showed only 3.1-log removal of FL particle, which was about 2-log lower than Membranes A and B despite their same category (LPRO). It is important to note that these three membranes have almost identical conductivity removal value ranging from 1.8 to 1.9-log (or 98.3% to 98.8%, respectively) **(Fig. 3)**, which is comparable to those obtained by LPRO membranes at full-scale water recycling plants (95–97%).[28] In addition, all four RO membrane elements in **Fig. 3** are standard 4 inch module with the same length (i.e. 1016 mm) and the diameter of permeate collection core tube (i.e. 19.1 mm) **(Fig. S6)**; thus, dimension is unlikely to be the cause of this

observed variation in log removal of FL particles. It is noted that Membrane C was supplied in a dry condition. Keeping RO membranes dry have an advantage for storage without being impacted by ambient temperature and bacterial growth. However, dry condition could alter the separation capacity of RO membrane, which may not be apparent for conductivity removal.

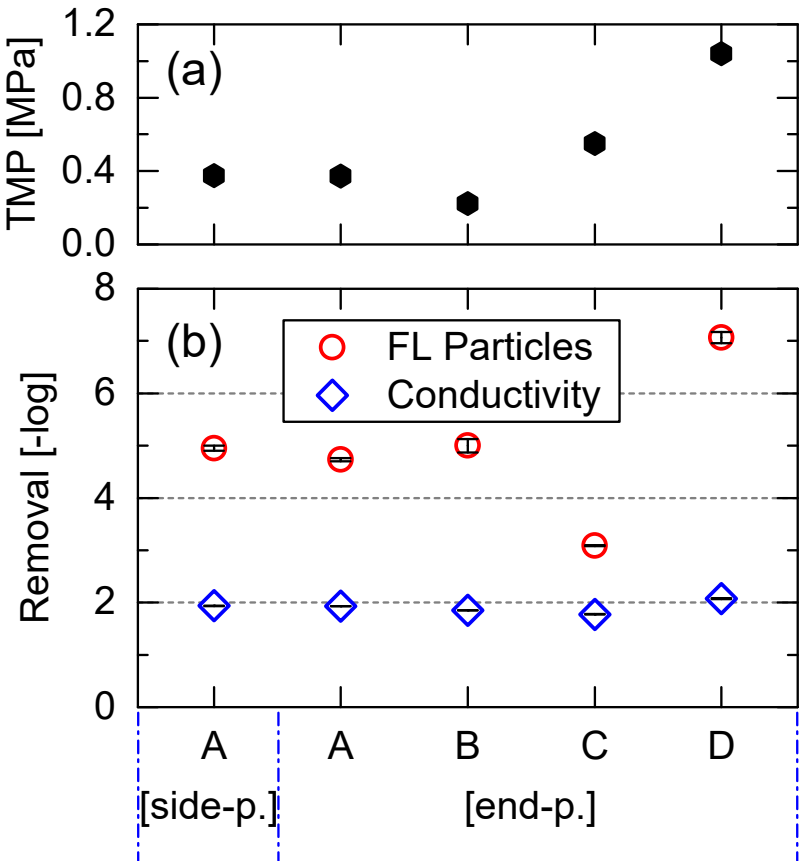


Fig. 3 – (a) TMP during test and (b) removal of FL particles and conductivity by four different RO membrane elements installed in a side-port (side-p.) or end-port (end-p.) pressure vessel. Permeate flux was maintained at 20 L/m^{2}h. The symbols and error bars are the average and standard deviation of two replicate samples collected at 30 and 60 min.

In an end-port vessel, Membrane D showed a considerably higher removal of FL particle (7.1-log) and slightly higher removal of conductivity (2.1-log) in comparison to Membrane A, B, and C. However, Membrane D has a low permeability and thus requires a higher TMP (1.04 MPa) to provide 20 L/(m^{2}h) permeate flux when compared to Membrane A, B, and C (TMP ranging from

220 0.22 to 0.55 MPa). Because Membrane D has to withstand high pressure difference between feed
221 and permeate sides, the physicochemical properties of RO membrane film can be different from
222 the other LPRO membranes. Although the underlying cause for this observed variation in FL
223 particle removal is still unclear, the results indicate that the selection of RO membrane type may
224 be an important factor to achieve high log removal of bacteria. In addition, the removal of FL
225 particles (bacterial surrogates) was confirmed to be far more sensitive than conductivity removal.
226 This indicates that rated salt rejection performance information typically provided by the
227 manufacturers is not a suitable indicator when it comes to the separation performance of highly
228 rejected constituents including FL particles. With regard to the incomplete removal of FL
229 particles by any of the RO membrane elements used here, it might be possible to attribute the
230 integrity of the overall membrane system integration (such as O-ring seal) to this observed
231 variation in removal of FL particle. This hypothesis will be further evaluated in the next section.

232 ### *3.2 O-ring seal*

233 To evaluate the contribution of O-ring sealing performance to the occurrence of bacterial passage
234 through RO membrane, the O-ring seal located at the interface between a pressure vessel and one
235 of the three LPRO membranes (Membrane A, B, and C) was reinforced by bonding with the
236 epoxy adhesive material. As a result, no observable changes in conductivity rejection were
237 recorded before and after the bonding **(Fig. 4)**. In contrast, a considerable increase in FL particle
238 removal after the bonding was observed for all three membranes by 0.2 to 0.4-log: from 5.0 to
239 5.4-log (Membrane A), from 5.0 to 5.2-log (Membrane B), and from 3.1 to 3.5-log (Membrane
240 C). These results confirm that by-pass through O-ring seal can be a major cause for the passage
241 of bacterial particles. In fact, another LPRO membrane manufactured without O-rings

(Membrane E), which has similar membrane properties to Membranes A and B **(Table S1)**, achieved a much higher removal (6.4-log) **(Fig. 4)**. Nevertheless, the removal of bacterial surrogates by Membrane E was not complete, indicating that, in addition to the O-ring, bacterial passage can occur through other locations within the membrane element and vessel.

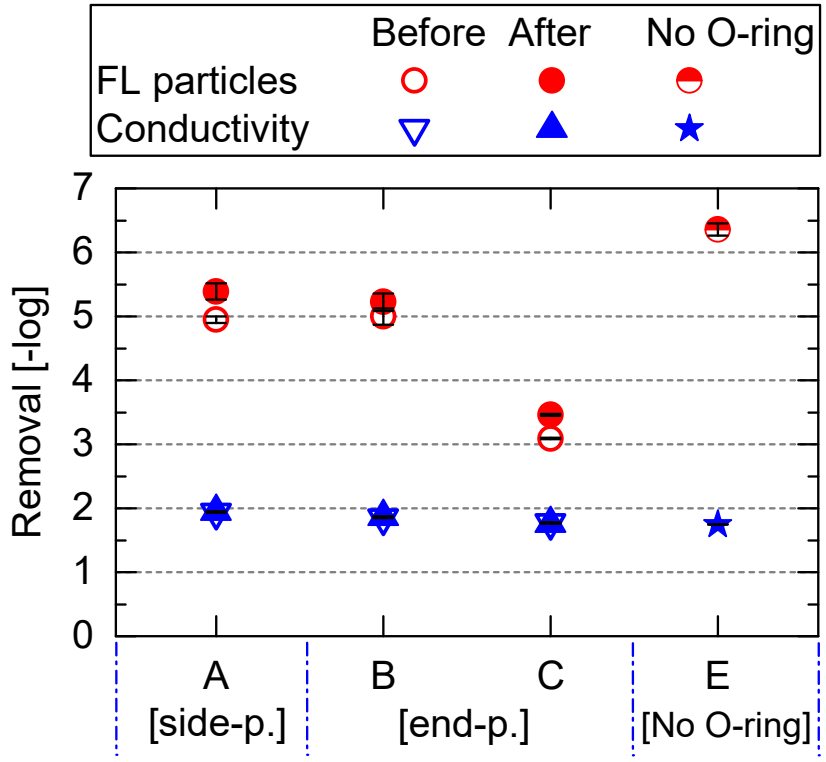


Fig. 4 – Removal of FL particle and conductivity by Membrane A, B and C before and after bonding O-ring seal and the O-ring free Membrane E. The symbols and error bars are the average and standard deviation of two replicate samples collected at 30 and 60 min.

The difference in the passage of bacteria through RO membrane element before and after bonding O-ring seal was further validated by counting bacterial number during the treatment of UF-treated wastewater. Using Membrane A element without the bonding of O-ring seal, bacterial counts in the permeate increased from the initial value of 23 counts/mL to 310 counts/mL when RO feed was replaced from RO filtered tap water to UF-treated wastewater **(Fig. 5)**. The level of increase in bacterial counts was considerably less for Membrane A with the bonding of O-ring

seal (from 14 counts/mL with RO filtered tap water to 79 counts/mL with UF filtered wastewater as the feed). In both cases, bacterial counts in RO permeate gradually decreased over time. The decreased bacterial counts in RO permeate occurred according to the reduction in bacterial counts in RO feed. The decreased bacterial counts in RO feed occurred probably due to the adsorption of bacteria in RO feed to the RO membrane surfaces and feed spacers, because the tests were conducted by recirculating the RO feed and permeate using a closed-loop RO system.

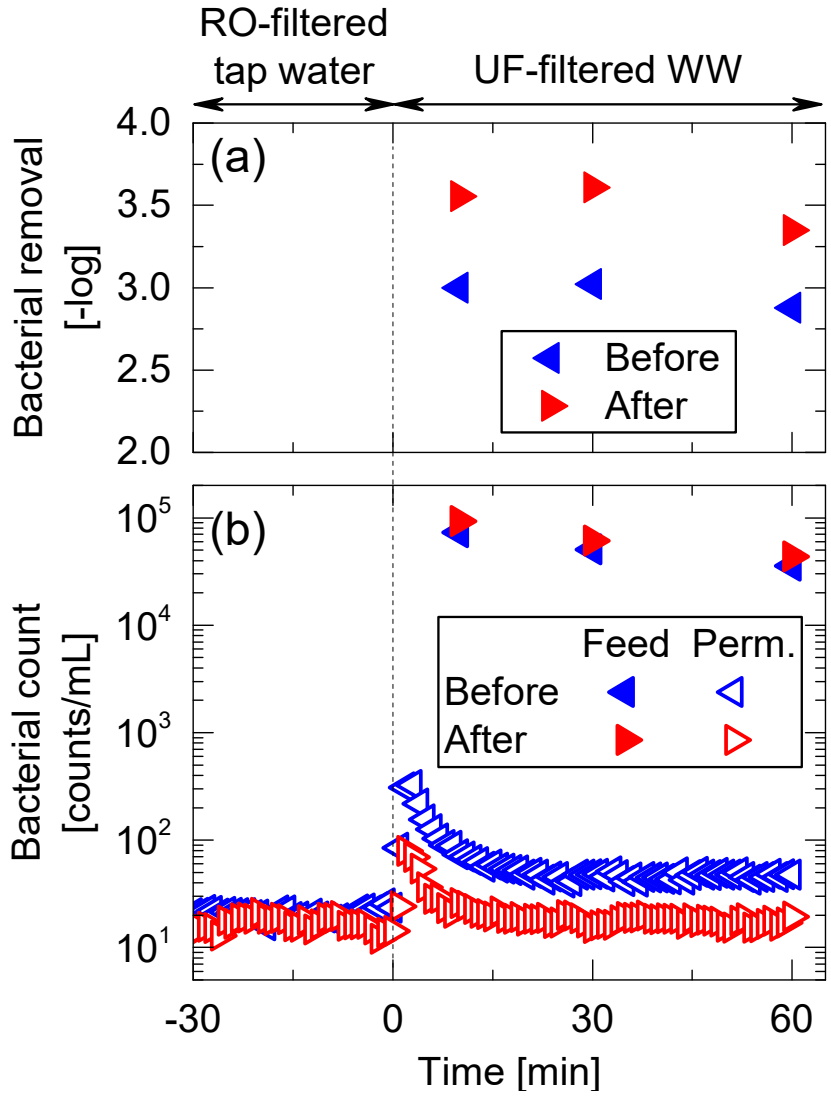


Fig. 5 – (a) Bacterial removal and (b) bacterial counts during the treatment of the RO-filtered tap water and UF-treated wastewater using Membrane A element (before and after bonding).

Similar to the tests using FL particle solutions, the manual sampling time (10, 30, and 60 min) was selected for RO feed sample collections to avoid determining the removal of bacteria under low bacterial concentrations in RO feed, which was expected to happen over filtration time. Thus, removal of bacteria was calculated using the samples collected at 10, 30 and 60 min. As a result, Membrane A with bonding O-ring seal showed a bacterial removal of 3.4–3.6-log, which was about 0.5-log higher than that by the Membrane A without bonding O-ring seal (2.9–3.0-log). The effect of bonding O-ring seal was also confirmed by manual bacterial count using epi-fluorescence microscopy: 2.5 to 2.9-log and 2.2 to 2.5-log removal by the Membrane A with and without O-ring seal bonding, respectively **(Table S2)**. The results here confirm that bacterial particle can pass through O-ring seal located at the end-cap of a pressure vessel. It is noted that bacterial counts by epi-fluorescent (DAPI) technique in the RO feed during the tests ranged from 1.7×10^5 to 7.6×10^5 counts/mL, which were comparable to those identified at up to 3.49×10^4 counts/mL in the RO feed (MF permeate) of a full-scale plant.[19] This indicates that the RO feed (i.e., MF- or UF-treated wastewater) typically contains high bacterial counts, which can be utilized for claiming high log removal value.

Enhanced bacterial removal value by 0.5-log through O-ring seal bonding is a considerable improvement in terms of bacterial concentration in RO permeate. The incomplete removal of bacterial surrogates and actual bacteria even after bonding O-ring seal indicates that passage of bacterial particles can also occur through the RO membrane and other locations (e.g. brine seal and membrane leaflet gluing) within the membrane element and vessel. These potential locations for the bacterial particle passage include the surface of flat sheet RO membrane (manufacturing defects or damage during membrane element assembly) and adhesive parts located on the edge of RO membrane sheets. Further evaluation of these mechanisms is planned in a future study.

3.3 Implications

Variation in bacterial passage through intact RO membrane elements and the contribution of intact O-ring seal to bacterial passage were identified in this investigation. This study used a single 4-inch RO membrane element in a pressure vessel, whereas RO processes for water recycling applications are typically equipped with 6–7 interconnected 8-inch RO membrane elements in each pressure vessel. Thus, the contribution of intact O-ring seal to bacterial passage in a full scale RO system could be even more profound. Moreover, the RO membranes and end-port pressure vessels used in this study were brand new, and the impact of aging of these components on bacterial passage has not been evaluated. Thus, a full-scale and long-term demonstration study is necessary to clarify the changes in bacterial passage through RO membrane over time.

Because LPRO membranes used in water recycling applications are designed for the removal of salts, the integrity of these RO membrane elements is mainly confirmed by undergoing a salt rejection test. However, in potable reuse it is preferable for RO membranes to have a high removal capability for bacteria. Accordingly, it is important to develop an RO membrane integrity test that can ensure high removal of bacteria-size particles. Based on the results obtained in this study, it is recommended that RO membrane manufacturers employ FL particle equivalent to bacteria in size for bacteria removal validation.

Real-time bacteriological counting technique can allow for continuous and online monitoring of bacterial particle counts in RO feed and permeate; thus, it has the potential for ensuring the RO membrane integrity higher than conventional indirect monitoring methods such as conductivity and TOC removal (i.e. 2-log). However, actual bacterial removal observed in this study was as

low as 3.1-log, indicating a potential margin for further improvement in the integrity of RO membrane process. Improved removal of bacteria by RO membrane up to 7-log removal may be possible through (a) the enhanced O-ring seal; (b) the enhanced protection of RO membrane sheet surface to avoid any damages during assembly; and (c) a better sealing between RO membrane element sheets. Overall, providing further understanding for the location of bacterial passage in a future study will allow for the achievement of the improved bacterial removal.

4 Conclusions

This study shows considerable variation in the removal of FL particles (used as bacterial surrogates) among three similar LPRO membranes in the range of 3.1 to 5.0-log. The reinforcement of O-ring sealing performance by bonding with epoxy adhesive materials improved the removal of bacterial surrogates by 0.2 to 0.4-log and the removal of bacteria in reclaimed water by approximately 0.5-log. Another LPRO membrane permanently stored in a pressure vessel without O-ring showed a higher removal of FL surrogate bacteria (6.4-log). The results indicate that O-ring seal is a major location for bacterial passage. The large variation in bacterial removal by these LPRO membranes was not reflected by conductivity removal (1.8–1.9-log), indicating that conductivity is not a suitable surrogate for monitoring the removal of bacteria by RO. In addition to O-ring seal, there may be other locations for bacterial passage in intact RO membrane elements. This study suggests that the improvement of O-ring sealing performance in an RO system can improve membrane integrity for bacterial removal.

5 Conflicts of interest

There are no conflicts to declare.

6 ACKNOWLEDGEMENT

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Supporting Information

Integrity of reverse osmosis membrane for removing bacteria: New insight into bacterial passage

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Table S1 – Specification of RO membrane elements.

Name	Model	Manufacturer	Membrane area [m^2]	Salt rejection [%]	Conditions during salt rejection measurement
A	ESPA2-LD-4040	Hydranautics	7.4	99.6	1500 ppm NaCl solution 1.03 MPa Applied Pressure 25 °C Operating Temperature 15% Permeate Recovery 6.5 - 7.0 pH Range
B	ESPA4-4040	Hydranautics	7.9	99.2	500 ppm NaCl solution 0.7 MPa Applied Pressure 25 °C Operating Temperature 15% Permeate Recovery 6.5 - 7.0 pH Range
C	BW30-4040	Dow/Filmtec	7.2	99.5	2000 ppm NaCl solution 1.55 MPa Applied Pressure 25 °C Operating Temperature 15% Permeate Recovery pH Range: Not available
D	HYDRApro-502-4040	Hydranautics	6.5	99.5	1500 ppm NaCl solution 1.55 MPa Applied Pressure 25 °C Operating Temperature 15% Permeate Recovery 6.5 - 7.0 pH Range
E	ESPA-FREE 3000L	Hydranautics	7.0	98.0	1500 ppm NaCl solution 1.05 MPa Applied Pressure 25 °C Operating Temperature 10–20% Permeate Recovery 6.5 - 7.5 pH Range

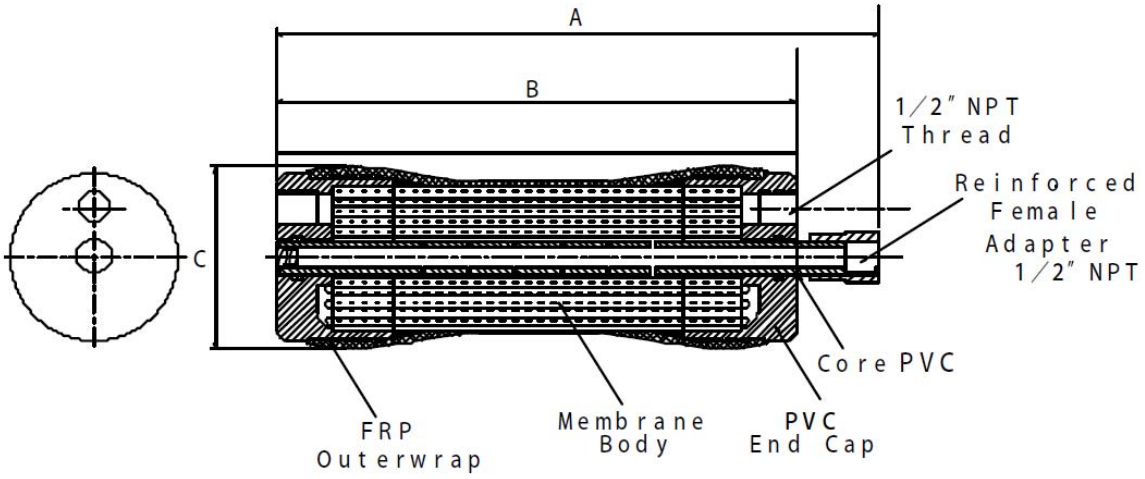


Fig. S1 – Schematic diagram of ESPA-FREE 3000L RO membrane.

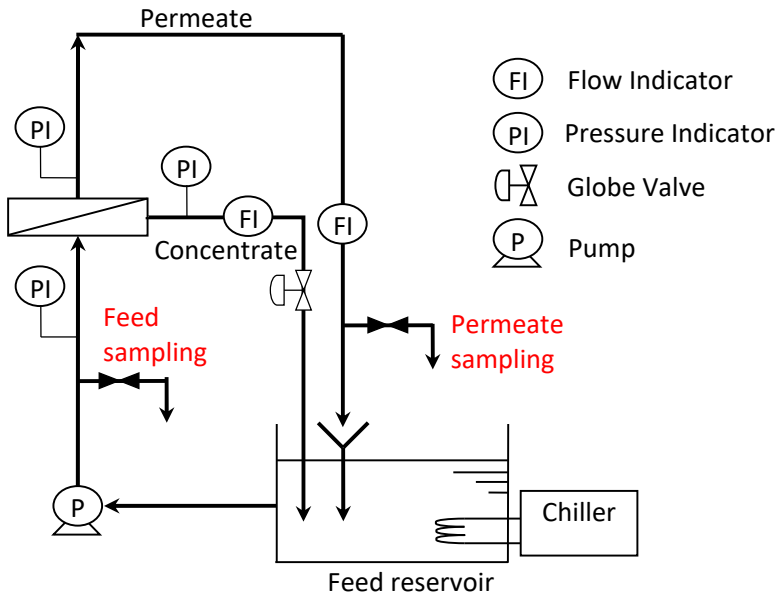
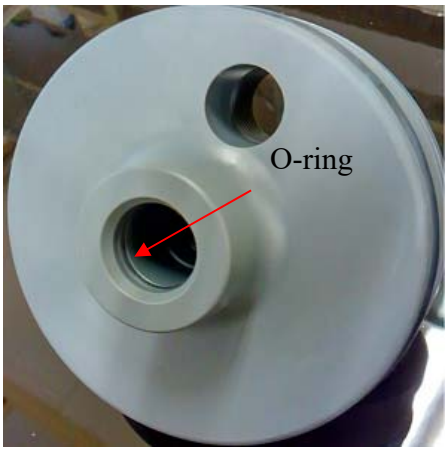


Fig. S2 – Schematic diagram of the RO treatment system.

(a) Side-port pressure vessel



(b) End-port pressure vessel

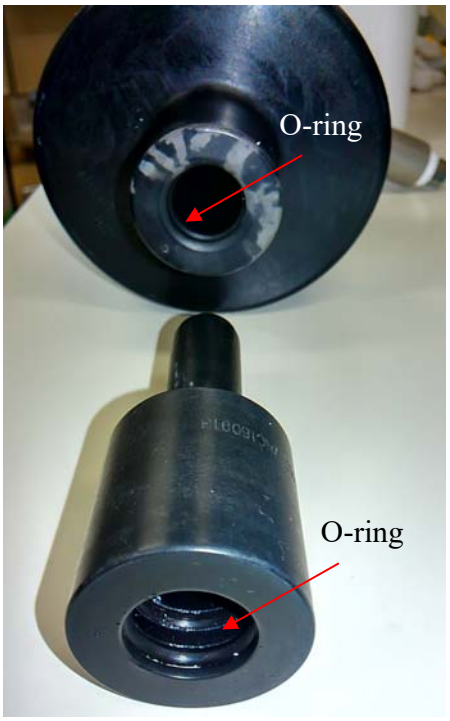


Fig. S3 – O-rings located at the end-cap of pressure vessels.

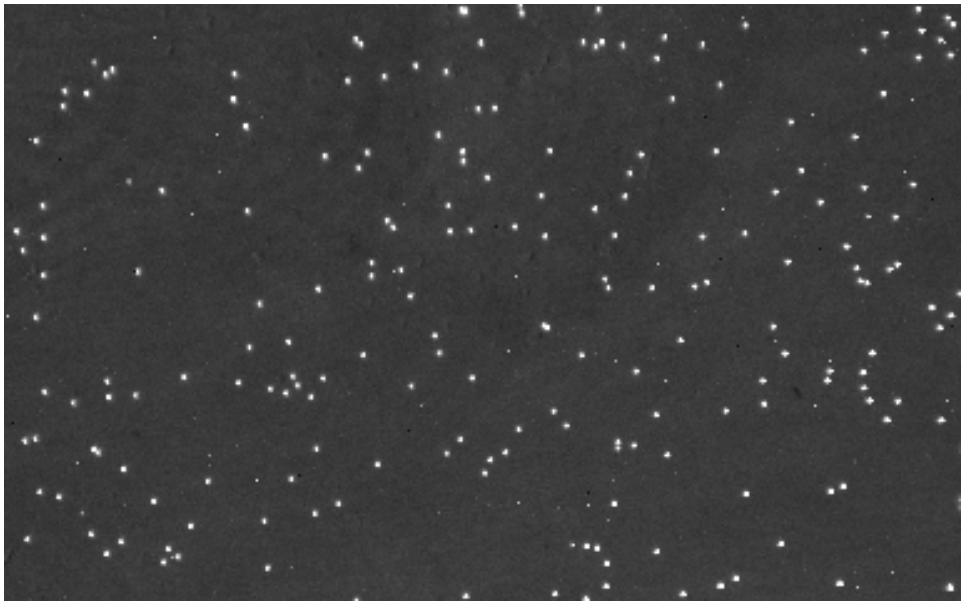
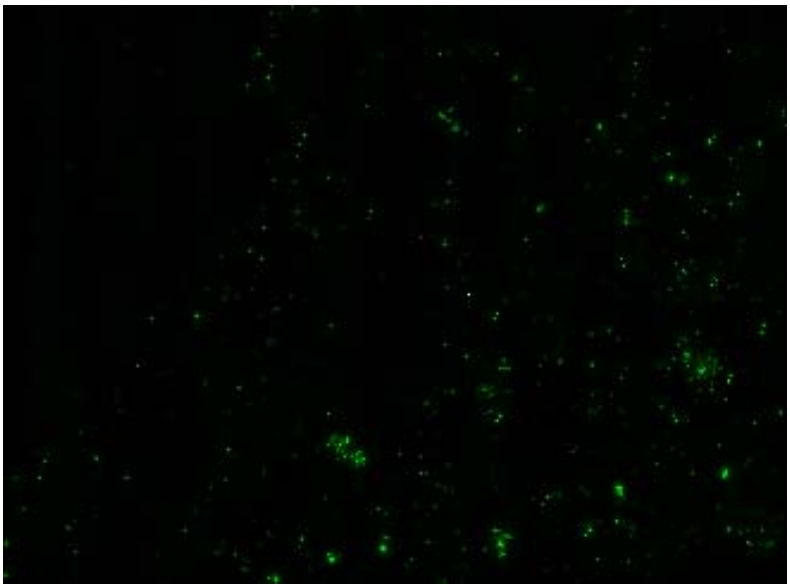


Fig. S4 – Typical image of FL particles in feedwater during experiment.

(a)



(b)

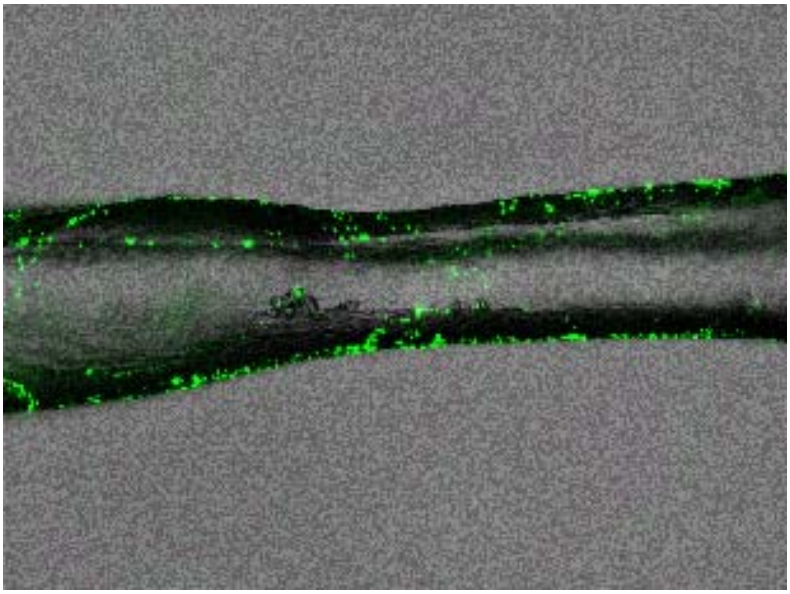


Fig. S5 – Images of FL particles deposited on (a) RO membrane surface and (b) feed spacer. They were obtained in the feed stream at 10 cm from the entrance of ESPA2 RO element after the test. Images were taken at x400 magnification using Fluorescence Microscope BZ-X800 (KEYENCE Co., Osaka, Japan).

	Membrane A, B, D	Membrane C
Outer diameter (mm)	19.1	19.1
Core tube extension, L_1 (mm)	25.9	26.7
Core tube extension, L_2 (mm)	27.2	26.7

Fig. S6 – Comparison in the size of core tube for Membranes A, B, C, and D.

Table S2 – Total bacterial counts by epi-fluorescence microscopy using DAPI (mean ± standard deviation, $n = 2$).

Time (min)		10	30	60
Before sealing	Feedwater (counts/mL)	409,656 ±17,595	298,818 ±8,109	174,414 ±33,007
	Permeate (counts/mL)	1687 ±60	884 ±35	1022 ±53
	Removal (%)	99.6	99.7	99.4
	Removal (-log)	2.39	2.53	2.23
After sealing	Feedwater (counts/mL)	755,864 ±23,052	594,237 ±18,504	316,935 ±32,028
	Permeate (counts/mL)	1002 ±47	966 ±85	1120 ±67
	Removal (%)	99.8	99.8	99.7
	Removal (-log)	2.88	2.79	2.45