Assessing bacterial infiltration through reverse osmosis membrane

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Abstract

The attenuation of pathogenic microorganisms in potable water reuse is critical to ensure recycled water safety. Thus, this study sought to identify bacterial communities capable of passing through a commercial reverse osmosis (RO) membrane as well as to characterize the passage of these bacteria through the membranes. Three-quarters of the bacteria in the RO permeate were found to belong to the Burkholderiaceae family, although this family only accounted for 0.2% of the RO feed (i.e., ultrafiltration-treated wastewater) bacterial composition. The infiltration routes of bacteria through the RO membranes was also evaluated using a unique approach—capturing bacteria-sized surrogates (i.e., 0.5 μm fluorescent (FL) microspheres) with a track-etched micro-filter after passing through the RO membrane. Our results demonstrated that a considerable number of FL particles passed through the membranes that were obtained from an RO membrane element. Overall, it was determined that certain bacterial families in wastewater could pass through the passage located in the entire surface of the RO membrane rather than in localized areas. Thus, this study highlights the need to reinforce RO membrane integrity in order to ensure the safety of recycled water for potable water reuse.

Keywords: Bacterial removal; membrane integrity; RO membrane; gene sequencing; potable water reuse.

1 Introduction

Pathogen control in potable water reuse is a critical public health measure (Pecson et al., 2017). Potable water reuse is accomplished by converting conventionally treated wastewater (i.e., secondary wastewater effluents) to highly purified drinking water. Thus, the attenuation of pathogenic microorganisms (e.g., virus, protozoa, and bacteria) through advanced wastewater treatment is critical. Advanced wastewater treatment systems encompass multiple barriers and treatment procedures such as microfiltration (MF) or ultrafiltration (UF), reverse osmosis (RO) membranes, advanced oxidation processes (AOP), and chlorine disinfection (Fujioka et al., 2012). Furthermore, the attenuation of pathogens in potable water reuse can be quantitatively managed by implementing a log reduction credit approach. For instance, California (USA) requires advanced wastewater treatment systems to meet a reduction value of 12-log, 10-log, and 10-log for virus, *Cryptosporidium*, and *Giardia*, respectively. Further, a 9-log reduction target has been suggested for total coliform bacteria for direct potable water reuse (NWRI, 2013).

Among advanced wastewater treatment processes, the RO membrane treatment has been undervalued with as little as a 2.0-log reduction credit value based on conservative surrogate indicators (e.g., electrical conductivity removal, which achieves up to 99% removal) that have widely been used to monitor and ensure RO membrane integrity (Tchobanoglous, 2015; WHO, 2017). Increasing the log reduction value of RO membranes could reduce the dependence on other bacterial removal processes, including AOP. For instance, recent advances in analytical technology (e.g. real-time bacteriological counters) could allow for continuous bacterial attenuation monitoring, thus potentially increasing the log reduction credit value for RO membranes (Fujioka et al., 2018b; Fujioka et al., 2019b).

In addition to improved monitoring techniques, the reliability of RO treatment for effective bacterial removal is also critical. To date, RO membrane reliability has been evaluated via integrity breaches caused by intentionally damaging the RO membrane (e.g., by creating pinholes on their surface) or the pressure vessel components (e.g., O-rings) (Antony et al., 2012; Kitis, Mehmet et al., 2003; Kitis, M. et al., 2003; Mi et al., 2004; Pype et al., 2016; Zhang et al., 2016). However, bacterial passage through intact (i.e., undamaged) RO membrane elements has been seldom addressed. Bacterial size (i.e., typically > 0.2 μm) is three orders of magnitude greater than the free-volume hole size of RO membranes (< 0.001 μm) (Fujioka et al., 2018c), meaning that in theory all bacteria should be rejected by RO membranes. However, many studies have identified high concentrations of bacteria in pilot- and full-scale RO system permeates (Ishida and Cooper, 2015; Kantor et al., 2019; Laurent et al., 1999; Liikanen et al., 2003; Miller et al., 2017; Park and Hu, 2010), indicating that RO is not an infallible barrier against bacteria. A recent study (Fujioka and Boivin, 2019) also confirmed the passage of particles through RO membranes using 0.5 μm fluorescent (FL) microspheres, which are surrogates similarly-sized to bacteria (e.g., *Escherichia coli* that has a diameter of 0.5 μm and a length of 2 μm).

A better understanding of bacterial passage through RO membranes could potentially lead to enhanced bacterial removal and improve the reliability of RO membranes. Our previous study (Fujioka et al., 2019a) found that bacteria could pass through intact O-ring seals, which connect multiple RO membrane elements and pressure vessel end caps. Our study also found that bacteria-sized surrogates (i.e., fluorescent microspheres) passed through different types of 4-inch RO membrane elements even after bonding the O-ring seals between the RO membrane element and the pressure vessel end-ports, indicating that bacterial passage can occur through the RO membranes themselves. Another recent study (Fujioka and Boivin, 2019) found that the integrity of RO membrane sheets for bacterial removal could be

compromised during their assembly processes. However, the previous study did not identify the bacterial communities that were passing through, nor did it explain how they passed through the RO membrane.

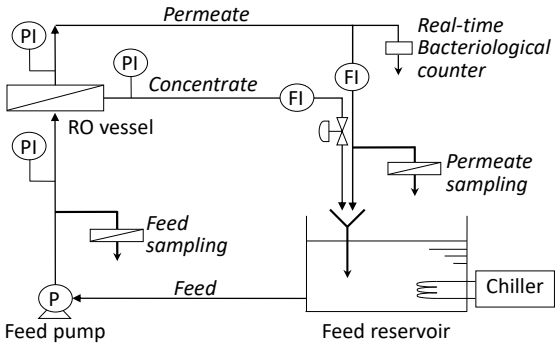
Therefore, the present study sought to identify bacterial communities that pass through RO membranes as well as their infiltration routes. Bacteria in RO feed and permeate were identified via bacterial cell counts by fluorescent staining and fluorescence microscopy, and 16S rRNA gene sequencing. In addition, their infiltration routes were evaluated using a unique approach—capturing bacteria-sized surrogates (i.e., 0.5 μm fluorescent microspheres) with a track-etched micro-filter after passing through the RO membrane.

2 Materials and methods

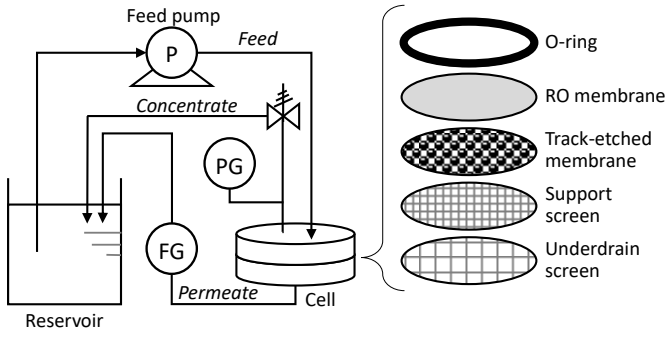
2.1 Membranes and treatment systems

2.1.1 Pilot-scale system

Bacterial infiltration through RO membranes was evaluated using a 4-inch spiral-wound polyamide (PA) composite RO membrane element (ESPA2-LD-4040, Hydranautics; Oceanside, CA, USA). The pilot-scale cross-flow RO system **(Fig. 1a)** consisted of a 4-inch end-port fiberglass pressure vessel (40E30N, Codeline/Pentair Water; Goa, India), a 65-L stainless steel reservoir, a high-pressure pump (25NED15Z, Nikuni Co., Ltd.; Kawasaki, Japan), digital flow indicators (FDM, Keyence Co.; Osaka, Japan), digital pressure indicators (GPM, Keyence Co.; Osaka, Japan), and a chiller unit (CA-1116A, Tokyo Rikakikai Co. Ltd.; Tokyo, Japan). The O-ring seal between the RO membrane element and the pressure vessel end-ports was bonded with adhesives to prevent bacteria from passing through the seal.



(a) Pilot-scale cross-flow RO system



(b) Bench-scale cross-flow RO system

Fig. 1 Diagrams of the (a) pilot-scale and (b) bench-scale RO treatment systems.

2.1.2 Bench-scale system

The location in the RO membrane sheets where bacterial passage occurred was examined in bench-scale experiments. Two different RO membrane conditions were examined herein: (a) RO membrane sheets without undergoing an RO element assembly process (i.e., hereafter referred to as "intact RO") and (b) RO membrane sheets after disassembling the RO element (i.e., hereafter referred to as "disassembled RO"). Previously inspected and certified polyamide composite RO membrane samples were supplied by an RO membrane manufacturer. Disassembled RO membrane samples were prepared by dismantling two RO membrane elements that had satisfied the manufacturer's specified performance. The bench-scale cross-flow RO system **(Fig. 1b)** was comprised of a 47-mm cross-flow stainless steel filter holder (XX440470, Merck; Tokyo, Japan), a dual plunger pump (KP-22, FLOM; Tokyo, Japan), a flow gauge, a pressure indicator, and a chiller unit (ACE-1100, Tokyo Rikakikai Co. Ltd.; Tokyo, Japan) **(Fig. 1a)**. To capture all particles passing through the RO membrane, a

115 track-etched polycarbonate MF filter with a uniform pore size of 0.2 μm (Merck; Tokyo,
116 Japan) was placed between the RO membrane and the support screen (i.e., permeate spacer).

117 *2.2 Experimental protocol*

118 2.2.1 Pilot-scale system

119 A pilot-scale test was conducted with 50 L of UF-treated wastewater, which was prepared by
120 filtering a secondary wastewater effluent with a UF membrane module (SFP-2860XP, Dow
121 Chemical; Midland, MI, USA). The UF membrane module had a membrane surface area of
122 51 m^2 and a nominal pore size of 0.03 μm. Secondary wastewater effluents (3.05 mS/cm
123 conductivity) were collected from a wastewater treatment plant that implemented primary
124 settling and activated sludge. Before testing, the pilot-scale RO system outflow was
125 disinfected using a commercial sterilant especially designed for such purpose (Minncare
126 Sterilant, Mar Cor Purification; Plymouth, MN, USA). Afterward, filtered tap water was
127 recirculated through the system for >12 hours until the RO treatment condition was stable.
128 Then, the water was replaced with the 50 L of UF-treated wastewater. The RO system was
129 then operated at a permeate flux of 16 L/m^2h, an feed temperature of 25 ± 0.5 °C, and a 14%
130 recovery (permeate and concentrate flow rate = 2.0 and 10 L/minute, respectively) for 27 h.
131 Both the RO permeate and concentrate were recirculated to the feed reservoir. RO feed and
132 permeate samples were periodically collected in a sterile polypropylene bottle and underwent
133 bacterial analysis soon thereafter. Additionally, RO feed and permeate conductivity was
134 analyzed using Orion Star™ A322 Conductivity meters (Thermo Fisher Scientific; Waltham,
135 MA, USA). Bacterial biomass for 16S rRNA gene sequencing was collected from the RO
136 feed and permeate by filtering the samples with a track-etched polycarbonate MF filter (0.2
137 μm pore size; Merck, Tokyo, Japan) at a flow rate of 2 and 150–200 mL/min, respectively.

2.2.2 Bench-scale system

Bench-scale tests were conducted using a stable fluorescent particle (FL; 0.50 μm diameter, diameter variation coefficient = 3%) solution containing Fluoresbrite® Yellow Green Carboxylate Microspheres (Polysciences, Inc.; Warrington, PA, USA). These stable FL particles were used as surrogates, as bacteria may occur on the intact RO membrane sheets and become the source of bacterial contamination. The FL solution was mixed into a 10 mM NaCl matrix. Before each test, the RO membrane was compacted to stabilize its performance by treating pure water at 0.6 MPa. Afterward, the water was replaced with 500 mL of solution containing 10 mM NaCl, and the pressure was reestablished to 0.6 MPa. Further, the FL stock solution was added to the RO feed solution at the concentration of approximately 2.2×10^7 particles/mL. The system was then operated at a constant permeate flux of 22 L/m^{2}h, a feed flow rate of 40 mL/min, and a feed temperature of 25 °C for 120 min. Following the filtration test, the track-etched polycarbonate MF membrane placed underneath the RO membrane was carefully removed and underwent fluorescence microscopy.

2.3 Analytical protocols

2.3.1 Bacterial counts

Intact and damaged bacterial counts were determined using a fluorescence microscope (BZ-X800, Keyence Co.; Osaka, Japan). Each 1-mL sample was stained with the LIVE/DEAD BacLight Bacterial Viability Kit (Thermo Fisher Scientific; Waltham, MA, USA) for 15 minutes in the dark at room temperature (i.e., approximately 23 °C). The staining kit contained two dyes: SYTO®9 and propidium. Afterward, 200 μL of the stained sample was passed through a 0.2 μm pore size track-etched polycarbonate MF filter (Merck; Tokyo, Japan) and analyzed using a green filter (excitation wavelength = 470 ± 40 nm, absorption

wavelength = 525 ± 50 nm) and a red filter (excitation wavelength = 545 ± 25 nm, absorption wavelength = 605 ± 70 nm).

Bacterial counts in RO permeate were also monitored using a real-time bacteriological counter (IMD-W^{TM}, Azbil Corporation, Tokyo, Japan). The real-time bacteriological counter is capable of counting bacterial particles in real time by measuring the intensity of scattered and fluorescent light that occur in response to the excitation light with a wavelength of 405 nm. Details of the instrument are provided elsewhere (Fujioka et al., 2018a).

2.3.2 Bacterial community analysis

Bacterial community analysis with 16S rRNA gene sequencing was conducted at Hokkaido System Science (Sapporo, Japan). Genomic DNA was extracted from the RO feed and permeate biomass samples using the Extrap Soil DNA Kit Plus ver.2 (Nippon Steel Eco-Tech Corporation; Tokyo, Japan). Using the extracted DNA samples, a first-stage PCR amplification of 16S rRNA genes was performed following the 16S Metagenomic Sequencing Library Preparation protocol provided by Illumina K.K. (Tokyo, Japan). The forward and reverse 16S rRNA amplicon polymerase chain reaction (PCR) primer pair used in this study were 341F (5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and 805R (5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C), respectively. The amplified products were then purified using AMPure XP beads, after which a second PCR (i.e., indexed PCR) was performed with dual indices and Illumina sequencing adapters using the Nextera XT Index Kit. The purification of the final library was performed with AMPure XP beads. The products were then sequenced (paired-end, 300 bp) using the MiSeq platform (Illumina K.K., Tokyo, Japan). The resulting sequences were initially processed by base calling, filtering, and trimming of each sequence to yield high-quality reads. Afterward,

sequence assembly and cluster generation were performed to create OTUs (i.e., operational taxonomic units). The QIIME (v1.8.0) bioinformatics pipeline was used for cluster generation. Analysis of the bacterial community was conducted for each OTU by searching homology in the 16S rRNA gene sequence database (Greengenes v13.8).

2.3.3 Membrane characterizations

FL particles that passed through RO membranes and deposited on the track-etched MF filter with a diameter of 47 mm were analyzed using a fluorescence microscope (BZ-X800, Keyence Co.; Osaka, Japan). The filter area was analyzed using a green filter as indicated above, but without the addition of stains. A 118 mm^2 (11.6 × 10.2 mm) area at the center of each filter was examined. FL particle counts were also determined automatically with specialized software (BZ-X800 Analyser, Keyence Co.; Osaka, Japan). Field emission - scanning electron microscope (FE-SEM) (S-4800, Hitachi, Japan) was used to obtain a cross-sectional image of ESPA2 RO membrane. Prior to the analysis, a membrane sample underwent freeze-fracturing, air drying, and coating with conductive material (Fujioka et al., 2018c).

3 Results

3.1 Passage through an RO element

3.1.1 Bacterial counts

Bacterial passage through the RO membrane element was quantitatively evaluated with manually stained and counted bacteria using fluorescence microscopy (i.e., intact and damaged bacterial counts). The UF-treated wastewater used in this study contained high bacterial concentrations of > 10^5 counts/mL **(Fig. 2)**. It is noted that although bacterial counts determined by heterotrophic plate counting method in RO feed (i.e., counts of colony-

forming bacteria) are typically found at < 100 CFU/mL, high concentrations of total bacterial cells determined by fluorescent staining in RO feed (e.g., 10^3–10^6 counts/mL) have been reported during pilot- or full-scale studies (Ishida and Cooper, 2015; Kantor et al., 2019; Miller et al., 2020). Thus, the UF-treated wastewater used in this study was not unusual. Pore size of the UF membrane element of this study (0.03 μm) was considerably smaller than typical bacterial size (> 0.2 μm). However, UF membrane element with a large membrane surface area has a potential of lesser membrane integrity than a small membrane samples that are typically at bench scale; thus, the log reduction of bacteria or bacteria-sized particles by UF membrane elements can range between 2 and 3-log (Hagen, 1998; Jacangelo et al., 1989).

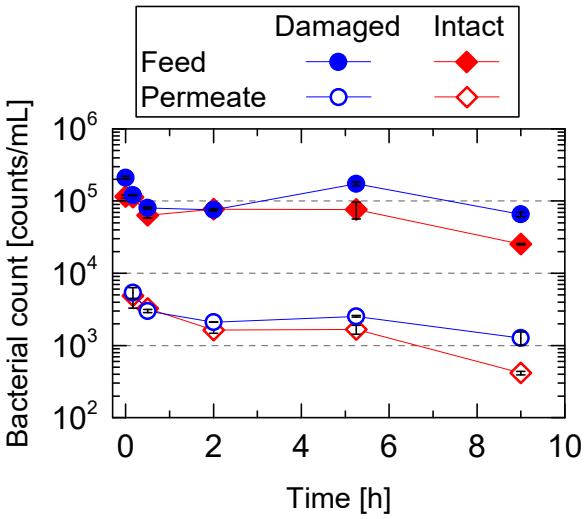


Fig. 2 Intact and damaged bacterial counts in the RO feed and permeate (transmembrane pressure of 270 kPa, permeate flux of 16 L/m^{2}h, feed temperature of 25±0.5 °C, and system recovery of 14%). The symbols and error bars of bacterial counts represent the average and ranges of duplicated analysis samples.

Throughout the pilot-scale RO treatment, the numbers of both intact and damaged bacteria in the RO feed and permeate gradually decreased **(Fig. 2)**. Bacterial removal value after 5 h were 1.7–1.8-log (i.e., 98% removal), which was similar to the conductivity rejection during the test (98.2–98.5%). It should be noted that the RO system was operated at a permeate flux of 16 L/m^{2}h, which was slightly lower than a typical permeate flux (20 L/m^{2}h), and a higher

permeate flux can enhance the rejection of constituents including salts (Wijmans and Baker, 1995). A recent pilot-scale study (Miller et al., 2020) also reported that nanofiltration and RO membranes achieved low bacterial removal values (1.7–1.8-log) when bacterial cells were counted based on fluorescent staining and flow cytometry. This indicated that approximately 2% of bacterial cells in the RO feed were continuously passing through the RO membrane regardless of whether they were intact or damaged. It is noted that high concentrations of bacteria in the RO permeate was detected during the first 30 min after changing water matrix (i.e. from pure water to UF-treated wastewater) **(Fig. 3)**. The online bacterial counting results also confirmed the reduction in bacterial counts determined by epifluorescence microscopy and continuous passage of bacteria through the RO membrane element.

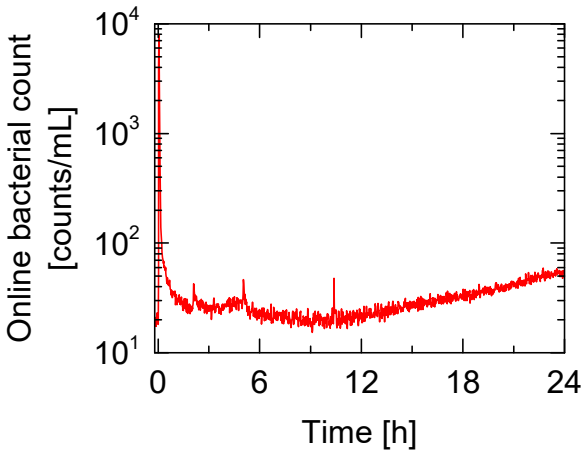


Fig. 3 Bacterial counts in the RO permeate that were online monitored using a real-time bacteriological counter during the pilot-scale test (flow rate of 10 mL/min).

3.1.2 Bacterial communities

Bacterial communities in both the RO input and permeate of the pilot-scale system were analyzed. Proteobacteria was found to be the major phylum (75%; **Fig. 4a**) in the RO feed, whereas it accounted for 97% of bacteria in the RO permeate **(Fig. 4b)**; compared to other phyla, Proteobacteria were the least affected by RO treatment. Proteobacteria have been frequently identified in RO permeates (Bereschenko et al., 2008; Stamps et al., 2018); therefore, the identification of bacterial passage mechanisms through RO filters could mainly

focus on this bacterial phylum. In this study, Proteobacteria in the RO permeate were further sub-classified. The class Betaproteobacteria accounted for the majority of bacterial classes in the RO permeate (84%), followed by the classes Alphaproteobacteria (13%) and Gammaproteobacteria (0.5%). It is worth noting that *E. Coli* (i.e., a typical bacterial indicator) belongs to the Gammaproteobacteria.

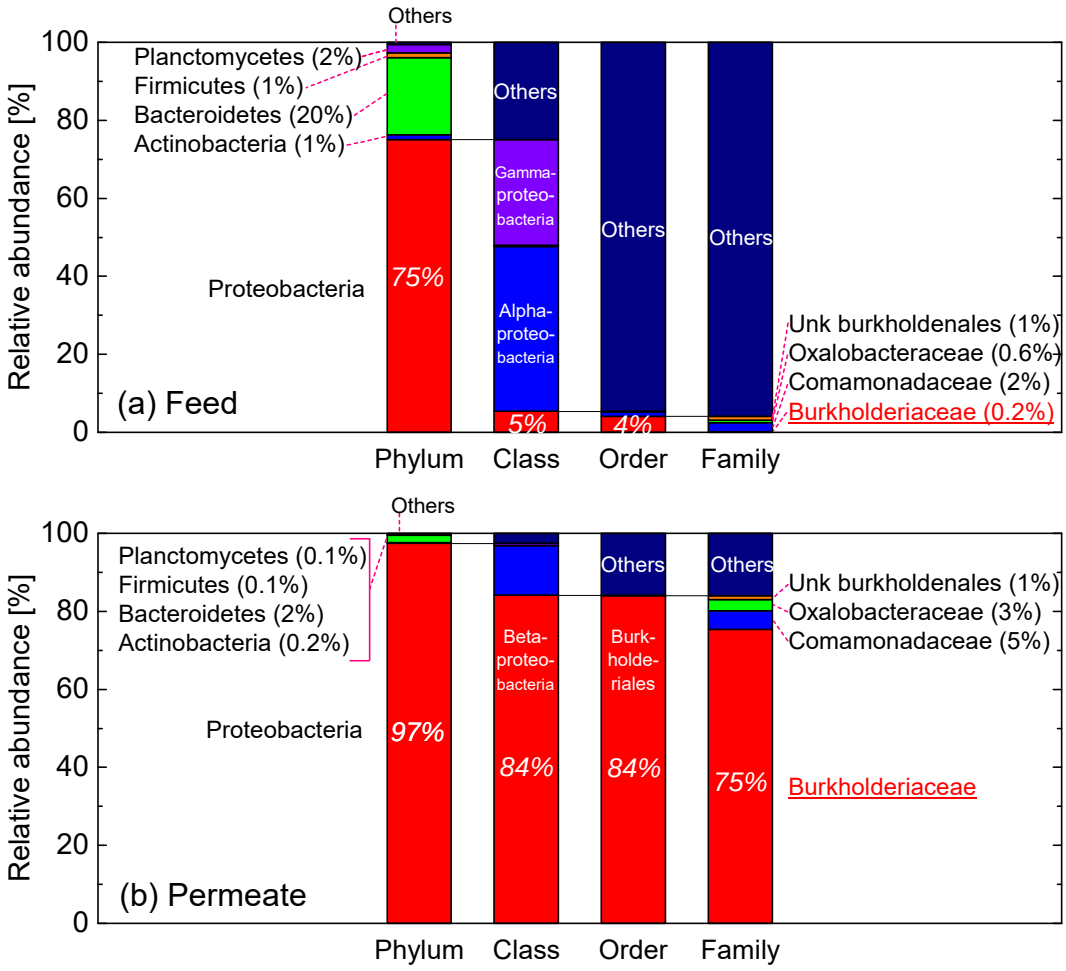


Fig. 4 Relative abundance of bacteria in the (a) RO feed and (b) permeate. Proteobacteria were further classified into major classes, orders, and families. "Unk" indicates unknown.

Almost all of the Betaproteobacteria found in the RO permeate were represented by the order Burkholderiales. The Burkholderiales included the families Burkholderiaceae (75%), Comamonadaceae (5%), and Oxalobacteraceae (3%). Notably, the Burkholderiaceae in wastewater are known to include the genera *Burkholderia*, *Pandoraea*, *Paraburkholderia*,

and *Ralstonia* (Stamps et al., 2018); however, their specific species were not identified in the present study. Three-quarters of the bacteria in the RO permeate belonged to the family Burkholderiaceae, although it accounted for only 0.2% of the bacterial composition in the RO feed. It should be noted that the contamination of microbial DNA that can occur through commonly used DNA extraction kits and other laboratory reagents influences the results of the samples containing a low microbial biomass (Salter et al., 2014); thus, the presented data needs careful assessment. Despite this limitation, the results of this study indicate that the Burkholderiaceae predominantly passed through RO membranes, despite representing a minor proportion of the RO feed bacterial community; however, most other bacterial families were effectively retained by the RO membrane.

Bacterial passage mechanisms were further explored in the literature. Burkholderiales (i.e., 85% of the RO permeate bacterial community) are relatively small and rod-shaped bacteria, which are presumably more likely to pass through membranes than other bacterial orders. For example, some species in this family have been reported to exhibit a diameter of 0.4–0.9 μm and a 1.0–2.8 μm length (Gao et al., 2018; Sahin et al., 2011). The Comamonadaceae, which accounted for 5% of the RO permeate bacterial community, include the species *Ramlibacter tataouinensis*, which exhibit a diameter of only 0.2 μm and a 3 μm length (Heulin et al., 2003). However, size estimation for nonculturable bacteria is a major challenge; thus, the size of many bacterial species is not well known. Bacteria smaller than 0.1 μm^3 in volume are classified as ultramicrobacteria (Liu et al., 2018; Silbaq, 2009). Moreover, bacteria size can vary greatly depending on the environment and growing conditions (Colwell and Grimes, 2000). Therefore, the mechanisms of bacterial passage through RO membranes cannot be solely attributed to differences in bacterial size. Rather, identification of the bacterial species in the RO permeate and their properties (e.g., shape and size) may be instrumental for the development of more effective RO membrane elements for bacterial removal.

3.2 Infiltration routes

Evaluations for the passage of bacteria in this study have been conducted based on bacterial concentrations in the RO permeate. Similarly, our previous study (Fujioka and Boivin, 2019) evaluated the removal of bacterial particle by intact and disassembled RO membranes by measuring the concentrations of bacteria-sized surrogates (i.e., FL particles) in the RO feed and permeate, and determined their removal values of 6.8 and 6.0-log, respectively. However, these concentration-based evaluations cannot provide the infiltration routes of bacteria through the RO membranes. Therefore, the specific infiltration routes of bacteria through RO membranes were evaluated using a unique approach—capturing the bacteria-sized surrogates on a 0.2 μm pore size MF filter, which was placed on the permeate side of the intact or disassembled RO membrane **(Fig. 1b)**. This study used stable and similarly-sized 0.5 μm FL microspheres as bacteria-sized surrogates to accurately quantify the particle passage without the influence of convective self-aggregation in the RO feed.

When the FL particle solution was treated with the disassembled RO membranes, a substantial number of FL particles (i.e., 532 ± 343 particles/cm^2; n = 4) were observed on the track-etched MF membrane **(Fig. 5a)**. For example, the captured FL particles formed a spot pattern on the left corner of the filter, and an enlarged representative image demonstrated that these FL particles pass through specific areas of the RO membranes. Contrary to the disassembled RO membranes, the intact RO membranes exhibited very high efficiency for FL particle removal **(Fig. 5b)**. A negligible number of FL particles were found to pass through the intact RO membranes (16 ± 1 particles/cm^2; n = 2), which was far less than the observed for the disassembled RO membranes. Many FL particles that passed through the disassembled RO membrane gathered in the same places and might have been counted as a single FL particle by the auto-counting software; thus, the measured FL counts are provided only as a qualitative analysis. Moreover, many FL particles appeared to stay behind or inside

of the RO membranes without being captured on the MF filter, which may have caused an FL particle count underestimation. Despite these limitations, the major difference in the number of FL particles that passed through the disassembled versus the intact RO membranes indicates that disassembled RO membranes are much more permeable to particles.

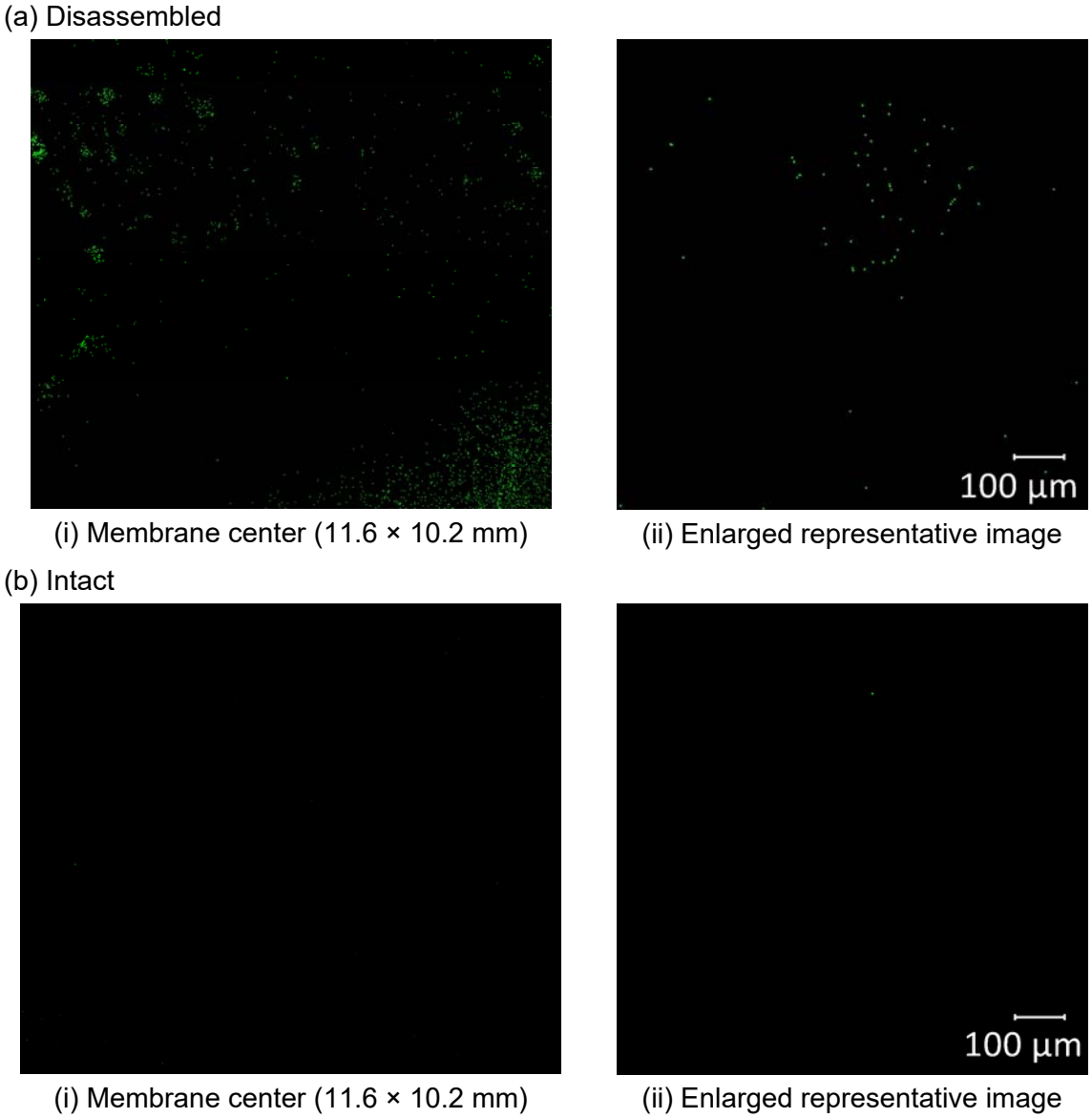


Fig. 5 Images of fluorescent (FL) particle captured on the track-etched microfiltration (MF) filter after treating the FL particle solution by (a) disassembled or (b) intact reverse osmosis (RO) membranes at a permeate flux of 22 L/m2h and a feed temperature of 25 °C for 120 min.

3.3 Implications

The passage of FL particles through the disassembled RO membrane sheet occurred likely due to damage caused by an element spacer, as suggested in our previous study (Fujioka and Boivin, 2019). The element spacer is placed between RO membranes in a spiral-wound element to maintain an opening in the RO feed channel **(Fig. 6)**. In fact, traces of a spacer were observed on the disassembled RO membrane surface, which is typically observed in disassembled RO membranes provided by many manufacturers. Typical commercial RO membranes have a thin polyamide (PA) skin layer (approximately 0.2–0.4 µm). The skin layer has a hollow interior of crumpled nodules and a ridge-and-valley structure (Fujioka et al., 2018c); thus, the actual thickness of its crumpled film can be as low as 30 nm (i.e., 0.03 µm) (Yan et al., 2015).

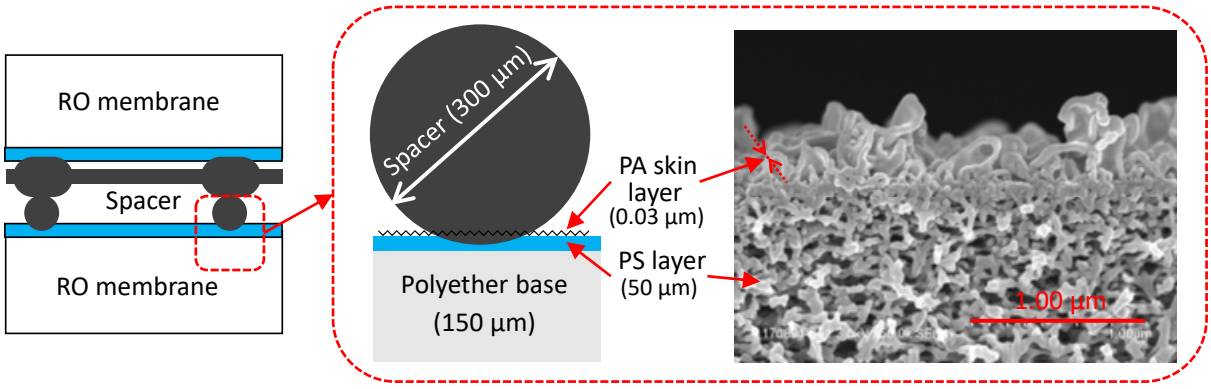


Fig. 6 Schematic cross-sectional images of a feed spacer and reverse osmosis (RO) membrane and a field emission - scanning electron microscope (FE-SEM) cross-sectional images of ESPA2 RO membrane.

The diameter of a feed spacer thread is approximately 300 µm, which is far greater than the thickness of the skin layer and UF polysulfone (PS) support layer **(Fig. 6)**. Therefore, the skin layer and UF support layer that contacted with the feed spacers can be damaged during the compression of an element assembling process, which may compromise the specific RO membrane regions with a spot pattern, as presented in **Fig. 5**. The compromised regions of the RO membrane surface can allow some small bacterial families in wastewater to

predominantly pass through. It should be noted that small constituents in wastewater may influence the passage of these small bacteria during long-term operation, since they may penetrate into the compromised regions and enhance bacterial removal. Because the compromised regions induced by feed spacers can occur throughout the RO membrane sheets, reinforcement of the entire RO membrane surface is required to improve bacterial removal performance. In addition, a future study will explore an approach that visualizes the compromised regions induced by feed spacers.

4 Conclusions

The results of this study using an epifluorescence microscopy and a real-time bacteriological counter indicated continuous passage of bacteria through an RO membrane element. Among bacteria in the UF-treated wastewater, Burkholderiaceae family predominantly passed through RO membrane, although this family only accounted for 0.2% of the RO feed bacterial composition. The results showed that disassembled RO membranes are more permeable to bacterial particles than intact RO membranes, because the thin polyamide skin layer can be damaged by contacting with the RO feed spacers. They are placed between RO membranes throughout the RO membrane element. Therefore, this study suggests that some specific bacterial families in wastewater are particularly capable of passing through the passage located in the entire surface of the RO membrane.

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