Abstract

Safety of potable reuse can be enhanced by improved water quality monitoring techniques for assessing water treatment processes. This study evaluated the efficacy of online bacterial counting for continuous monitoring of reverse osmosis (RO) membranes to remove bacteria using real-time bacteriological commercial counters and an on-site pilot-scale RO system. Prior to on-site assessments, the online bacterial counting was verified by comparing the measurement of fluorescent particles in water with flow cytometry. During a seven day pilot test of RO treatment at a water reclamation plant, online bacterial counts in RO permeate were monitored below 15 counts/mL; whereas the bacterial counts in RO feed water were approximately 2,500 to 10,000 counts/mL. Removal rates of bacterial counts ranged from 2.6 to 3.1-log (average = 2.9-log) by continuously monitoring bacterial removal. This is greater than a 2-log reduction frequently determined using other water quality surrogates (i.e., electrical conductivity). Overall, the continuous monitoring of bacteria in RO feed and permeate can be implemented without the addition of chemicals to provide near real-time bacterial counts to measure their reduction after RO treatment. This can be developed for continuous performance monitoring of the RO process, providing greater assurance of microbial water quality after RO treatment.

Keywords: real-time bacterial count; flow cytometer; integrity monitoring; potable water reuse; RO membrane.

1 INTRODUCTION

Microbial risk assessment and management of recycled water is important to ensure public health protection (WHO, 2017). This is particularly relevant in potable reuse, which recycles treated wastewater into drinking water. To ensure the safety of recycled water, recent potable reuse projects have used quantitative microbial risk management, in which the pathogenic risks are minimized by reduction of pathogens through multiple sequential water treatment processes such as microfiltration (MF) or ultrafiltration (UF), reverse osmosis (RO), advanced oxidation process (AOP), and disinfection (Amoueyan et al., 2017; Pecson et al., 2017; Shi et al., 2018). As a result, the required log reduction value of viruses and protozoa (e.g. *Cryptosporidium* and *Giardia*) in California is 12-log and 10-log, respectively. Enteric bacterial pathogens have also become important control targets (Barker et al., 2013; Mosher, 2016; NRMMC et al., 2008). For example, a 9-log total coliform reduction has been recommended for direct potable reuse (NWRI, 2013).

Among advanced water treatment processes, RO treatment plays a key role in removing viruses and low molecular weight constituents such as dissolved salts and trace organic chemicals. However, the reliability for pathogen removal by RO treatment is generally considered to be low and unknown because pathogen monitoring techniques that provide instantaneous results to assess RO membrane performance are lacking (WHO, 2017). In fact, most water reuse projects in the United States have employed two conservative but readily monitored surrogate substances for continuous RO integrity monitoring: total organic carbon (TOC) and electrical conductivity (EC), which account for a maximum of 2-log (i.e. 99%) reduction (Tchobanoglous, 2015; Zhang et al., 2016). Compared to surrogate substances, direct microbial counting techniques, in particular for bacteria, have a potential for improved membrane integrity monitoring. A previous

full-scale study (Ishida and Cooper, 2015) reported that bacterial counts in RO feed (i.e. MF effluent) with epi-fluorescence microscopy were 400–3,500,000 counts/mL, which suggests an over 2-log reduction of bacteria after treatment is achievable. Thus, this study specifically focused on an automated, on-line method for counting bacteria in RO feed and resolving their reduction in the RO permeate. To measure bacterial counts in water treatment systems flow cytometry has been used extensively in recent years due to its high speed and versatility (Ou et al., 2017; Prest et al., 2014; Van Nevel et al., 2017; Whitton et al., 2018). Bacterial count using a flow cytometer is based on particle counting and nucleic acid staining. Flow cytometry can also be used with real-time monitoring (Besmer et al., 2017).

Other bacterial counting techniques that have recently emerged are real-time bacteriological counting instruments without cell staining (Højris et al., 2016; Højris et al., 2018; Pepper and Snyder, 2016). The real-time bacteriological counter used in this study is based on two key technologies, particle counting and auto-fluorescence detection, which can count bacterial particles in real time without any chemical additions or attenuation of the sample. The real-time bacterial counters can detect bacterial auto-fluorescence by a sensor that detects and distinguishes bacterial particles from abiotic particles. Real-time bacterial counters are capable of monitoring bacterial counts in RO permeate; these measurements can be important in monitoring biofilm formation potential, which can be enhanced by dissolved organic matter in RO permeate (Liang et al., 2014). For the first time, a recent study by Fujioka et al., (2018) demonstrated real-time variations in bacterial counts in RO feed and permeate using an automated real-time bacteriological counter. The study overcame analytical challenges associated with RO feed, which typically contains high concentrations of humic-like substances that interfere with the analysis by adopting an online dilution technique. However, the efficacy of this technique on-site

66 remains unclear because the assessment was conducted in a laboratory using a closed loop
67 system that can cause unusual variations in bacterial communities in RO feed. Moreover, the
68 reliability of the real-time technique is an additional uncertainty, as it has not been assessed with
69 other bacterial counting techniques such as flow cytometry.

70 In this study, we evaluated the applicability of a real-time, chemical-free method for counting
71 bacteria to monitor the RO performance as it applies to bacterial removal. The study covered two
72 major objectives: (a) verification of the real-time bacterial counting technique, and (b) on-site
73 assessment of bacterial removal by pilot-scale RO system. Real-time bacterial counting method
74 was verified using stable surrogate substances (i.e. fluorescent microspheres) and actual bacteria
75 to compare the results with both flow cytometry and epi-fluorescence microscopy. Pilot-scale
76 assessment was conducted by continuously tracking the variation in bacterial counts and their
77 removal by RO using the real-time bacterial counting technique. In this study, we present an
78 application of on-line monitoring of bacterial counts for assessing RO performance and the water
79 quality in real-time.

80 # 2 MATERIALS AND METHODS

81 ## *2.1 Analytical methods*

82 Two real-time bacteriological counters (IMD-W^{TM}) provided by Azbil Corporation (Tokyo,
83 Japan) were used. The analytical system is based on two key technologies (particle size and auto-
84 fluorescence detections) (Fujioka et al., 2018). For the excitation (Ex) light (wavelength = 405
85 nm), (a) scattered light for counting particles in water and (b) two auto-fluorescence emission
86 (Em) lights (wavelength = 410–450 and 490–530 nm) from riboflavin and nicotinamide adenine
87 dinucleotide - hydrogen (NADH) in bacteria are detected, which allows for counting bacterial

cells. The two auto-fluorescent Em lights can identify and exclude non-bacterial particles from auto-fluorescents from bacteria because interferents such as silicon and PTFE particles produce peaks at lower wavelengths (wavelength = 410–450 nm) compared to bacterial particles that produce peaks at higher wavelengths (wavelength = 490–530 nm) (Scott, 2017).

Flow cytometric analysis was performed using a flow cytometer (BD Accuri® C6, BD Biosciences, San Jose, CA, USA). The instrument was set to irradiate the sample excitation light (wavelength = 488 nm), and emission was detected through an optical filter (533/30 nm). According to the manufacturer, the minimum and maximum detection limits of the instrument are 100 and 9.6×10^7 counts/mL, respectively. Epi-fluorescence counts were determined using a fluorescence microscope (Rapisco, Shibasaki, Inc., Chichibu, Japan). Both viable and nonviable bacterial cells in RO feed and permeate samples were stained with 4'-6-diamidino-2-phenylindole (DAPI) dye (Thermo Fisher Scientific, Waltham, MA, USA) and counted as total direct bacterial counts **(Text S1)**. For staining bacterial cells in treated wastewater, SYBR Green I nucleic acid gel stain (Takara Bio, Kusatsu, Japan) was used at 1% concentration. Standard plate count agar (PCA) method was also used to determine viable bacterial counts in water **(Text S1)**.

2.2 Instrumental assessment protocols

The online bacterial counts as determined by using the real-time bacteriological counter was verified by counting the number of stable surrogate substances (i.e., fluorescent particles) in Milli-Q water at four different concentrations and comparing the results with epi-fluorescence microscopy (referred to as direct fluorescent particle counts) and flow cytometry. Four different concentrations were determined to fit the detection range for flow cytometry. The fluorescent

particles used were SPHERO™ Yellow Fluorescent Particles (low Intensity, 0.7–0.9 μm) supplied by Spherotech (Lake Forest, IL, USA). The excitation (Em) and emission (Ex) spectra of fluorescent particles are covered by the excitation light and emission detection of the three instruments **(Fig. S1)**.

2.3 Assessment using a pilot-scale RO treatment system

This study used a pilot-scale RO treatment system **(Fig. S2)** located in a water recycling demonstration plant in Kokura (Fukuoka, Japan) (Takabatake et al., 2013). A brand new 4-in. spiral wound RO membrane element with the surface area of 7.43 m^2 (ESPA2-LD-4040, Hydranautics/Nitto, Oceanside, CA, USA) was installed in a pilot-scale cross-flow RO filtration system. The RO feed was the effluent from a membrane bioreactor (MBR) without disinfection (e.g. chloramination). The NH_4^+, NO_3^+ and chemical oxygen demand of the MBR effluent during the test was determined as 0.2, 10, and 6–8 mg/L, respectively. The pilot-scale RO system was operated at a target permeate flux of 19–20 L/m^2h. Prior to counting the bacterial cells, the RO feed underwent a 50-fold dilution using RO permeate that was subsequently filtered with MF filter **(Text S2)**. RO feed water and permeate conductivity was manually analyzed using a conductivity meter (Orion Star™ A325, Thermo Fisher Scientific, MA, USA).

3 RESULTS AND DISCUSSION

3.1 Instrumental evaluation

The capability of online bacterial counting as determined by using a real-time bacteriological counter was assessed by counting the number of fluorescent particles in water and comparing the numbers with fluorescent particle counts based on epi-fluorescent microscopy (referred to as

direct fluorescent particle counts) and flow cytometry. The fluorescent particles that have high dispersiveness, high stability, and high fluorescence emission intensity without staining are suitable as non-bacterial surrogates. This is because the emission intensity of bacteria is influenced by bacterial species, instrument settings, pre-treatment methods, and staining protocols. Direct fluorescent particle counts by epi-fluorescent microscopy using microscopic analysis can be more accurate than other methods; thus, direct fluorescent particle counts were used for comparison with other analytical methods. A high correlation was identified between direct fluorescent particle counts and the two analytical methods (i.e. real-time bacteriological counter and flow cytometry) with Pearson correlation coefficient (r) = 0.996 and 0.999, respectively **(Fig. 1)**. The particle counts at the lowest concentration as determined by all three methods resulted in a variation between 667–2,700 counts/mL **(Fig. 1)**. The online and flow cytometry counts were 53% greater than and 62% less than direct fluorescent particle counts, respectively. Less variation was identified in the sample with the second lowest concentration, in the range of 5,610–7,700 counts/mL. The online and flow cytometry counts were 37% and 19% greater than direct fluorescent particle counts, respectively. The sample with the highest concentration also showed variation ranging from 272,828 to 628,444 counts/mL. The online and flow cytometry counts were 18% less than and 89% greater than direct fluorescent particle counts, respectively. At high bacterial concentrations, the fluorescent particle counts from the online bacterial counter were less than the direct fluorescent particle counts. Nevertheless, the high correlation between online and direct fluorescent particle counts indicates that the online bacterial counter can be calibrated with those by epi-fluorescence microscopy.

[Fig. 1]

Online bacterial counts in RO feed water (i.e. MBR effluent) were also compared with bacterial counts by flow cytometry and epi-fluorescent microscopy using DAPI stain (referred to as total direct bacterial counts). The resulting bacterial counts by flow cytometry (4,101±993 counts/mL, n = 3) were comparable with online bacterial counts (3,800 ± 260 counts/mL, n = 3). However, total direct bacterial counts of the RO feed water was approximately one magnitude greater (100,363 ± 2,742 counts/mL, n =3). A similar level of difference (one magnitude) in bacterial counts in wastewater between real-time bacterial counter and total direct bacterial count has also been identified by others (Højris et al., 2018). In treated wastewater, measured total direct bacterial counts can be higher when compared with other methods because MBR effluent can contain fine particles (smaller than membrane pore size) including bacteria. Other visual counting techniques using microscopy will count all bacterial particles of all sizes as long as they are visible. In contrast, both flow cytometry and online bacterial counter used in this study detect particle sizes from 0.5 to 40 μm and >0.2 μm, respectively. Therefore, they are unlikely to measure the smaller bacterial particles and their fluorescence. The differences in counts among the three methods may also be due to the different principles used to identify bacteria. Both dead and alive bacteria are identified by epi-fluorescence microscopy using DAPI. Similarly, flow cytometry using SYBR Green I, which has recently been applied to drinking water applications, measures both dead and alive bacteria. In contrast, bacterial counts by real-time bacteriological counter rely on the auto-fluorescence light emitted from riboflavin and NADH, this includes auto-fluorescence of dead or stressed (i.e. injured) cells that are likely to emit lower fluorescence. Overall, more studies incorporating various controls that use different species, sizes, ATP, and culturability (e.g. death/alive) of bacteria) in wastewater are necessary for fair comparison among these techniques.

3.2 Pilot-scale assessment of online bacterial counting

The variation in bacterial numbers and their reduction due to RO treatment was examined by continuously monitoring the bacterial counts before and after RO treatment at a pilot-scale RO system. The pilot-scale test began with a 17 hr stabilization phase (from −17 to 0 hr., **Fig. S3**); this was performed to minimize the concentration of bacteria present in the RO system (e.g. sampling line, connectors and back side of RO membrane). During that period, online bacterial counts in RO permeate reduced from 30 to 10 counts/mL. Another real-time bacteriological counter installed for RO feed measured only the dilution water (MF-filtered RO permeate), resulting in very low bacterial counts, <1 counts/mL.

[Fig. 2]

Over the course of a 7-day test, online bacterial counts in RO feed varied from 2,500 to 10,000 counts/mL **(Fig. 2)**. During the first day (0–24 hr), online bacterial counts in RO feed remained at approximately 2,500 counts/mL. A considerable increase in online bacterial count was observed, up to 6,000 counts/mL, before the RO system halted (from 26 to 29 hr) due to the MBR system undergoing a relaxation phase (from approximately 25 to 27 hr) and the MBR-treated wastewater in the RO buffer was depleted. After the restart of the RO system, online bacterial counts in the RO feed reached approximately 9,000 counts/mL and gradually decreased. The increase occurred likely due to irregular flow through the MBR or RO buffer tanks; this could result in disturbances of sediments and bacteria being deposited on the bottom of the buffer tanks. From 121 to 130 hr, a relatively long peak was identified in RO feed. This peak occurred from 10 am to 7 pm on Monday; thus, it was likely caused by changes in industrial activities over the weekend. In contrast to the RO feed, online bacterial counts in RO permeate

198 remained low (<15 counts/mL) throughout the experiment **(Fig. 2)**. The results indicate that
199 bacteria can be identified at relatively low concentrations in RO permeate, which can be used to
200 determine online removal rates at extremely low concentrations. Detection of bacteria in RO
201 permeate or after nanofiltration has been reported in many previous studies (Ishida and Cooper,
202 2015; Miller et al., 2017; Palma et al., 2016; Park and Hu, 2010). However, the cause of the
203 occurrence (e.g. pass through fittings of RO membrane or bacterial growth) has not been
204 identified (Liu et al., 2013; Pype et al., 2016).

205 RO feed and permeate samples were also manually collected for the analysis of other water
206 quality parameters. Through epi-fluorescence microscopy using DAPI, total direct bacterial
207 counts were identified in RO feed (26,000–68,000 counts/mL) and in RO permeate (505–1,000
208 counts/mL) **(Table S1)**, all within the range of those previously published using DAPI (bacterial
209 counts in RO feed and permeate = 400–3,500,000 and 272–1,232 counts/mL, respectively)
210 (Ishida and Cooper, 2015). In treated wastewater (i.e. RO feed and permeate), total direct
211 bacterial counts **(Table S1)** were consistently greater than one magnitude when compared to
212 online bacterial measurements **(Fig. 2)**. Plate counts showed lower concentration of bacterial
213 counts than those determined by using online bacteriological counters **(Table S1)**. Viable
214 bacterial counts as determined by PCA were low in RO feed (16–49 CFU/mL) and were non-
215 detected in RO permeate, except at 23 and 30 hrs. It should be noted that majority of bacterial
216 species do not form a colony; thus, the number of bacteria by plate counting methods can be
217 underestimated. Conductivity in RO feed and permeate remained stable in the range of 1016–
218 1275 and 6–9 µS/cm, respectively. Removal rates calculated using conductivity was
219 approximately 2.1–2.2-log, which is consistent with a previous study using a full-scale RO
220 system (Pecson et al., 2017).

3.3 Separation performance analysis

During the pilot scale test shown in **Fig. 2**, bacterial rejection calculated by online bacterial counts remained stable within the range of 99.40–99.98%, which corresponds to approximate removal rates of 2.3–3.7-log **(Fig. S4)**. The performance distribution curve of online bacterial count removal was relatively sharp with the average and standard deviation of 2.9-log and 0.2-log, respectively; this is greater than the reduction determined by electrical conductivity in this study (i.e. 2.1–2.2-log) **(Fig. 3)**. The interruption of the RO system occurred from 26 to 29 hr that did not significantly influence the removal of bacteria. More importantly, between 48–110 hr, there were many peaks in the RO feed that reached as high as 30,000 counts/mL; this increased the removal to approximately 3.7-log. The occurrence of short but frequent peaks may be associated with some release of bacteria attached on the inner surface of the sampling pipe. To avoid overestimating, the counts attained during the peaks should be removed when evaluated for integrity monitoring. Overall, the range of bacterial reduction after excluding the peaks over the course of seven day was determined as 2.6–3.1-log.

[Fig. 3]

The results from this study indicate that an online bacterial counter can be applied to continuously monitor bacterial reduction by RO treatment. This can enable proactive measures for RO treatment processes and water quality measurements. In addition, higher bacterial removal rates as determined by continuous online bacterial counting than by low-cost conductivity measurement of the RO process can provide greater evidence towards higher log removal credit and improved trust and confidence in the water quality of the RO product water. The versatility of an online bacterial counter and its ability to consistently and accurately monitor

243 bacterial removal rates should be further validated with various water matrices and multiple RO
244 membrane elements run under different conditions (e.g. after fouling, chemical cleaning and
245 aging) at full scale. In addition, in future studies the reduction in bacterial counts can also be
246 considered as a surrogate indicator for the overall reduction in the microbial population
247 (excluding viruses). For example, the removal of protozoa (e.g. *cryptosporidium* and *giardia*) by
248 RO membrane is fundamentally governed by size exclusion, because protozoa (>1 μm) are
249 generally one order larger than bacteria (>0.2 μm). Thus, online bacteriological counters can also
250 be used to monitor the concentration and the removal of microorganisms such as protozoa.

251 # 4 CONCLUSIONS

252 This study evaluated the efficacy of a real-time bacterial counter to continuously monitor a pilot-
253 scale RO system for bacterial removal. This study demonstrated that the concentration of
254 fluorescent particles (surrogates) by online bacterial counter correlated well with direct
255 fluorescent particle counts. In addition, online bacterial counts of an actual RO feed water was
256 found to be comparable to bacterial counts by flow cytometry. During a seven day pilot-scale test
257 of RO treatment, online monitoring using real-time bacteriological counters continuously
258 provided bacterial counts in RO feed and permeate without any chemical additions. The range of
259 bacterial reduction over the course of seven day was determined as 2.6–3.1-log; this is greater
260 than the widely accepted removal rates for bacteria (2-log), that are generally based on reduction
261 in electrical conductivity (EC). Higher bacterial removal rates through online bacterial counting
262 can provide higher credibility for bacterial removal by the RO process, which can help to gain
263 improved confidence in water quality. Overall, in this study, the continuous monitoring of
264 bacteria in RO feed and permeate provided near real-time removal rates for bacteria, which
265 otherwise cannot be achieved.

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363

FIGURES

Fig. 1 – Fluorescent particle counts determined by online bacteriological counter and flow cytometry as a function of direct fluorescent particle counts determined by epi-fluorescence microscopy. The online bacterial counts recovered for 3 min were averaged. The plots for flow cytometry and epi-fluorescence microscopy were averaged and ranges are provided (n = 3).

Fig. 2 – Bacterial counts of (a) RO feed and (b) RO permeate every 5 min during RO treatment of MBR effluent at the pilot scale (transmembrane pressure = 0.7 MPa).

Fig. 3 – Process performance probability distribution of bacterial removal rates as determined by online bacterial counters.

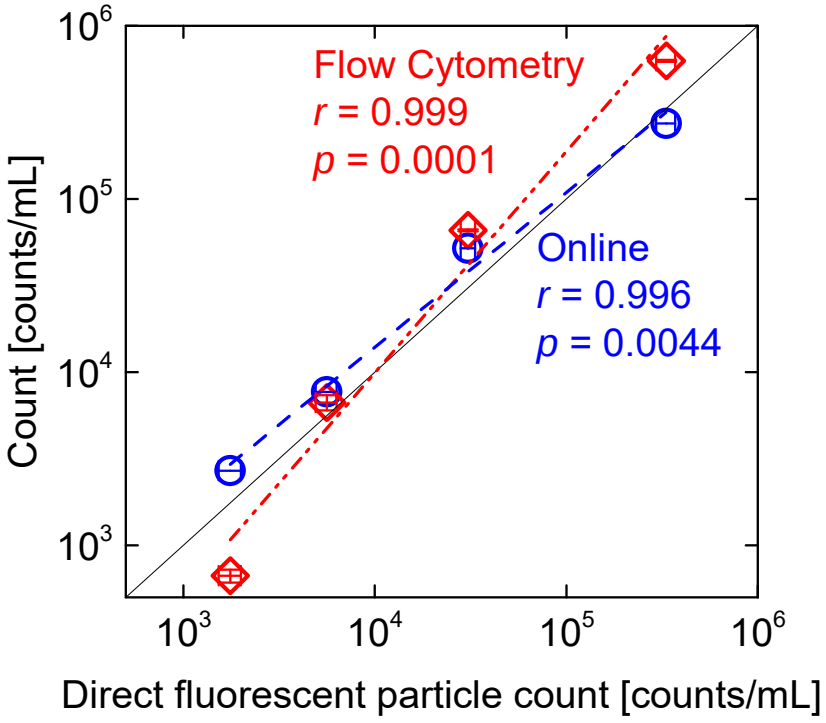
Flow Cytometry
r = 0.999
p = 0.0001
Online
r = 0.996
p = 0.0044
Count [counts/mL]
Direct fluorescent particle count [counts/mL]
10^3
10^4
10^5
10^6

Fig. 1

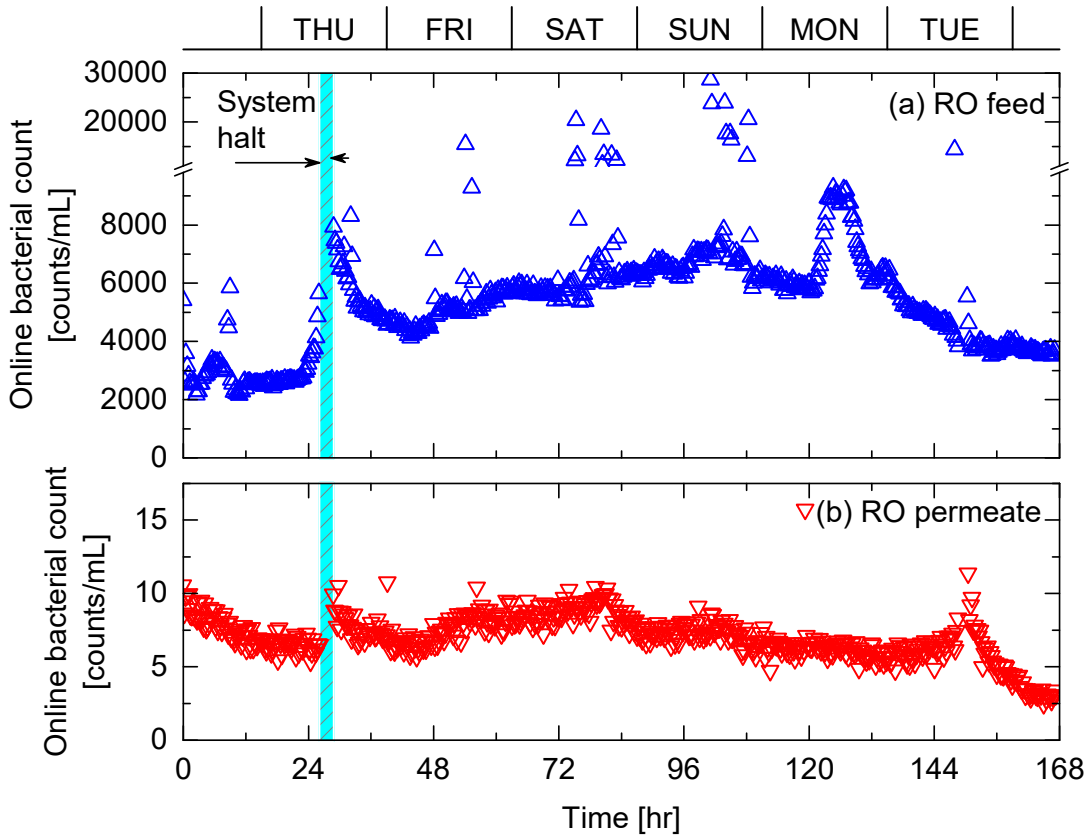
THU
FRI
SAT
SUN
MON
TUE
System
halt
(a) RO feed
Online bacterial count
[counts/mL]
30000
20000
8000
6000
4000
2000
0
(b) RO permeate
Online bacterial count
[counts/mL]
15
10
5
0
0
24
48
72
96
120
144
168
Time [hr]

Fig. 2

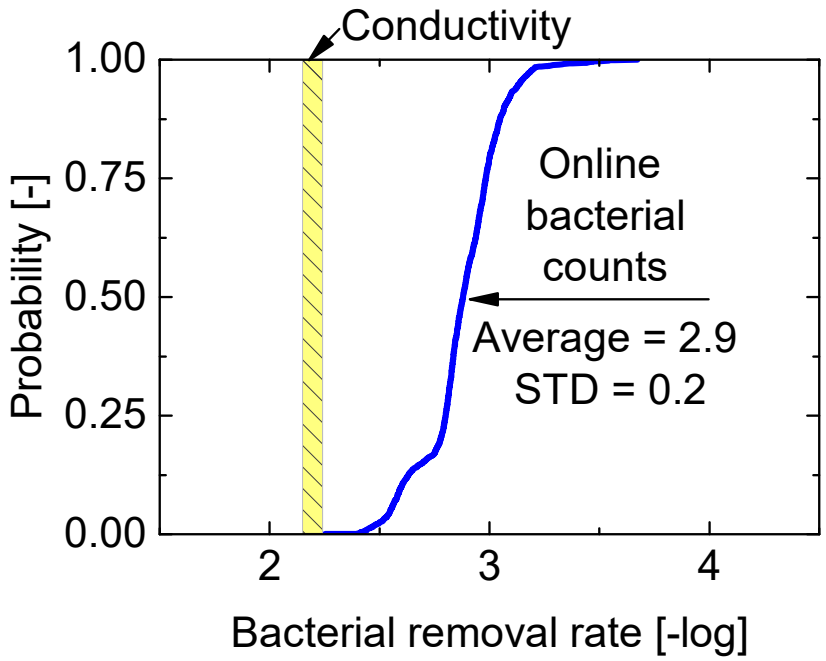
Conductivity
1.00
0.75
0.50
0.25
0.00
Probability [-]
Online
bacterial
counts
Average = 2.9
STD = 0.2
2
3
4
Bacterial removal rate [-log]

Fig. 3

Supporting Information

Assessment of online bacterial particle counts for monitoring the performance of reverse osmosis membrane process in potable reuse

Takahiro Fujioka,[1,*] Ryo Makabe,[2] Naomichi Mori,[3] Shane A. Snyder,[4,5] Menu Leddy,[6]

[1]*Water and Environmental Engineering, Graduate School of Engineering, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*

[2]*R&D division, Kyowakiden Industry Co.,Ltd., 10-2 Kawaguchi-machi, Nagasaki 852-8108, Japan*

[3]*Water Reuse Promotion Center, 4-5 Nihonbashiyokoyamachō, Chuo, Tokyo 103-0003, Japan*

[4]*Nanyang Environment & Water Research Institute (NEWRI), Nanyang Technological University (NTU), 1 Cleantech Loop, CleanTech One, Singapore 637141, Singapore*

[5]*Department of Chemical and Environmental Engineering, University of Arizona, 1133 E. James E. Rogers Way, Harshbarger 108, Tucson, AZ 85721-0011, USA*

[6]*Orange County Water District, 18700 Ward Street, Fountain Valley, CA 92708, USA*

* Corresponding author: Takahiro Fujioka, Email: tfujioka@nagasaki-u.ac.jp, Tel: +81 095 819 2695, Fax: +81 95 819 2620

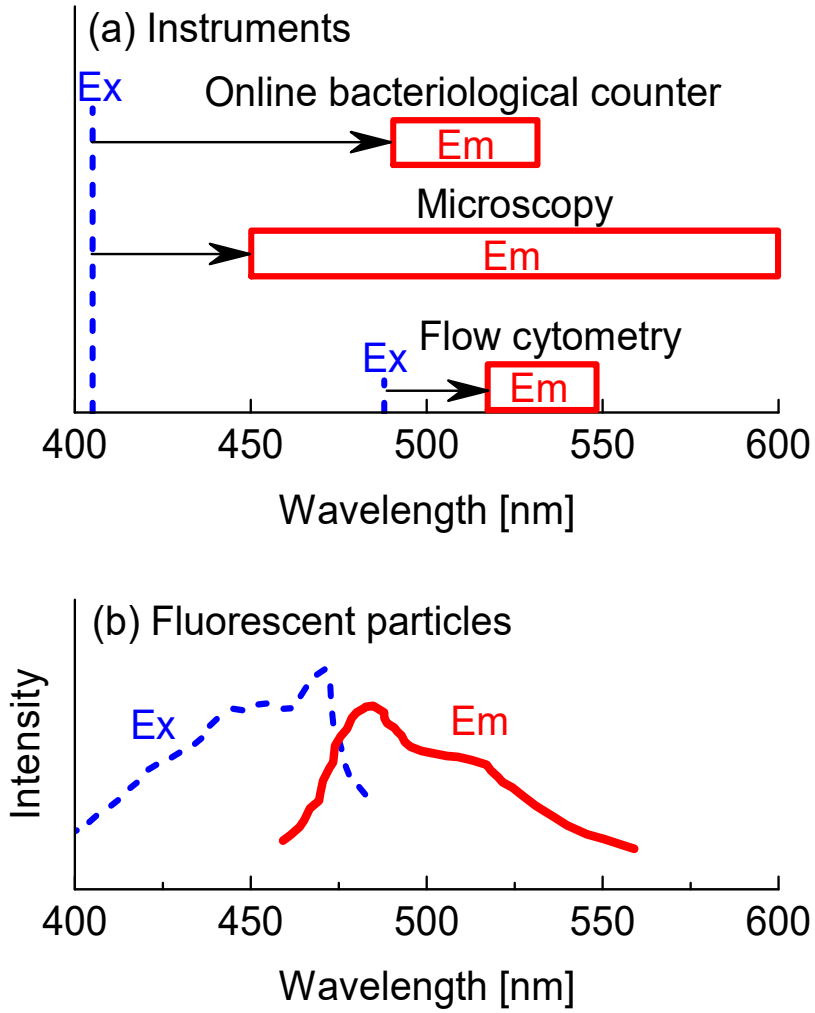

Fig. S1 – Excitation and emission wavelength of (a) three instruments, and (b) SPHERO™ Yellow Fluorescent Particles.

Text S1 – Epi-fluorescence counts and standard plate count agar method.

To measure fluorescent particle counts, 1 mL of each sample was filtered using a track-etched polycarbonate MF membrane with 0.22 μm pore size and a filter diameter of 13 mm (Meric, Tokyo, Japan) and their number deposited on 40% of the filter surface area was measured. No staining was applied prior to the analysis. Total number of both viable and nonviable bacteria (i.e. total direct bacterial counts) was analyzed for RO feed and RO permeate samples. RO permeate sample did not undergo any dilution. RO feed sample was first diluted 50 times using microfiltration (MF) membrane-treated pure water. Thereafter, 1 mL of each sample was filtered using a track-etched polycarbonate MF membrane with 0.22 μm pore size and a filter diameter of 13 mm (Meric, Tokyo, Japan). After 10 min staining, bacterial number deposited on 40% of the filter surface area was measured and total direct bacterial count was expressed in counts/mL.

Standard plate count agar (PCA) method was used to determine viable bacterial counts in water, expressed as colony-forming unit (CFU). Each sample (1 mL) was added to about 15 mL sterile standard plate count agar medium (Nissui Pharmaceutical Co., Tokyo, Japan) at a temperature of 45–50°C and mixed. Thereafter, they were poured into sterile Petri dishes and left to stand until solidified. Following, the plates were incubated at 20–25 °C for 22–26 hrs.

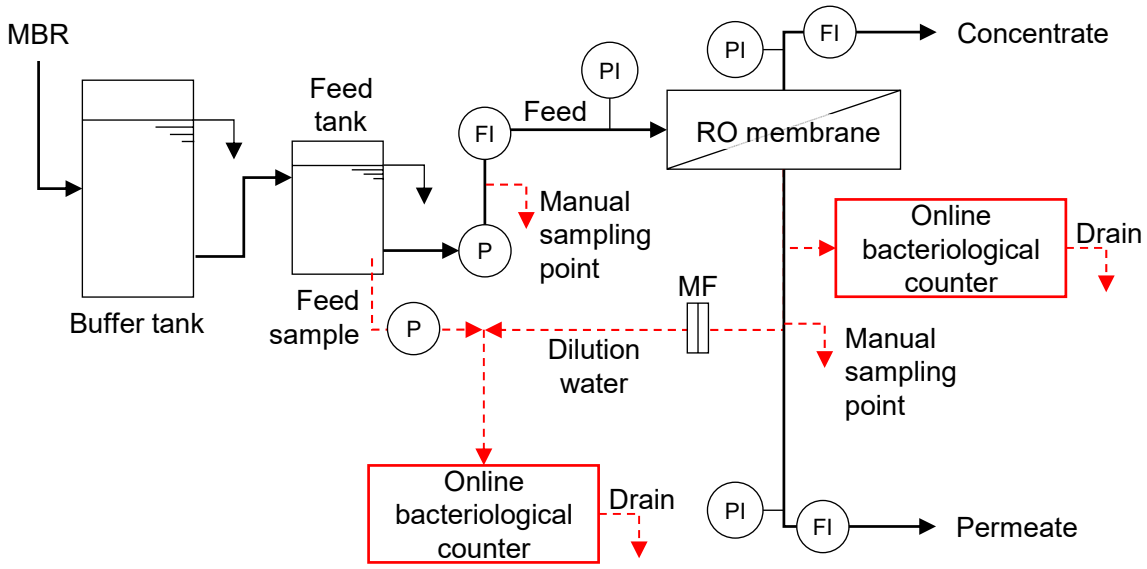

Fig. S2 – Flow chart of the pilot-scale RO system. PI = pressure indicator; FI = flow indicator; TI = temperature indicator; P = pump; MF = microfilter.

Text S2 – Pilot-scale RO treatment and online analysis.

The pilot-scale RO system holding one RO element was operated at a target permeate flux of 19–20 L/m^{2}h with system recovery of 15% (permeate and concentrate flow rate = 2.5 and 16 L/min, respectively). It is noted that full-scale water recycling RO systems typically comprise of three stages with system recovery of up to 85%. Each RO membrane contained in a pressure vessel achieves less than 15% water recovery. Sampling tubes for two real-time bacteriological counters were located at the RO feed tank and RO permeate stream. RO feed was designed to undergo 50-fold dilution prior to the real-time analysis, because RO feed contains high concentrations of organics (e.g. humic-like substances), that are not counted as particles but can exceed the capacity of auto-fluorescence detectors of the real-time bacteriological counter.

The pilot-scale testing started with a stabilization phase for the first 20 hrs (from −20 to 0 hr). During the stabilization phase, the online bacteriological counter located in the RO feed stream received a flow of dilution water to ensure the cleanness of pre-filtered RO water. The dilution water was prepared online by filtering RO permeate with a 0.2 µm nominal pore size micro-filter (Minisart® syringe filter, Sartorius, Göttingen, Germany). From 0 hr, RO feed was fed at 0.2 mL/min to the dilution water using a dual plunger pump (KP-22, FLOM, Tokyo, Japan), which accounted for a 50-fold dilution. In contrast, online bacteriological counter in the RO permeate stream continuously received RO permeate at 10 mL/min. During the course of one week of testing, the pilot-scale RO system was halted at 26–29 hr, this is standard operating procedure to minimize membrane fouling and is often referred as relaxation period.

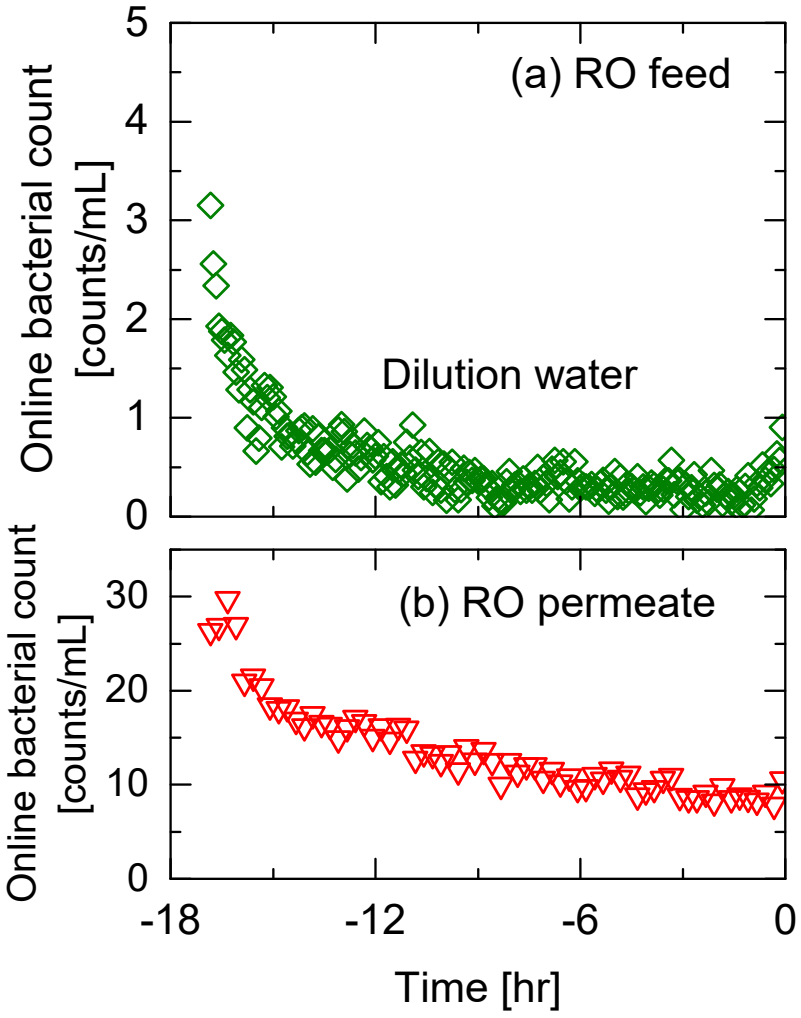

Fig. S3 – Online bacterial counts of (a) the dilution water in RO feed stream and (b) RO permeate before RO treatment of MBR effluent at the pilot scale (transmembrane pressure = 0.7 MPa).

Table S1 – Manual sampling data during the pilot-scale test.

Time	[h]	0.2	6	23	30	47	83
Total direct bacterial count							
Feed	[counts/mL]	26,620	32,120	43,140	67,890	68,410	34,280
Permeate	[counts/mL]	680	715	790	1000	690	505
PCA							
Feed	[CFU/mL]	33	23	49	16	27	49
Permeate	[CFU/mL]	0	0	0	7	9	0
Electrical conductivity							
Feed	[µS/cm]	1148	1275	1191	1225	1264	1016
Permeate	[µS/cm]	8.1	8.9	7.7	8.0	8.7	5.8
Feed temperature	[°C]	18.3	19.6	18.9	20.1	19.3	20

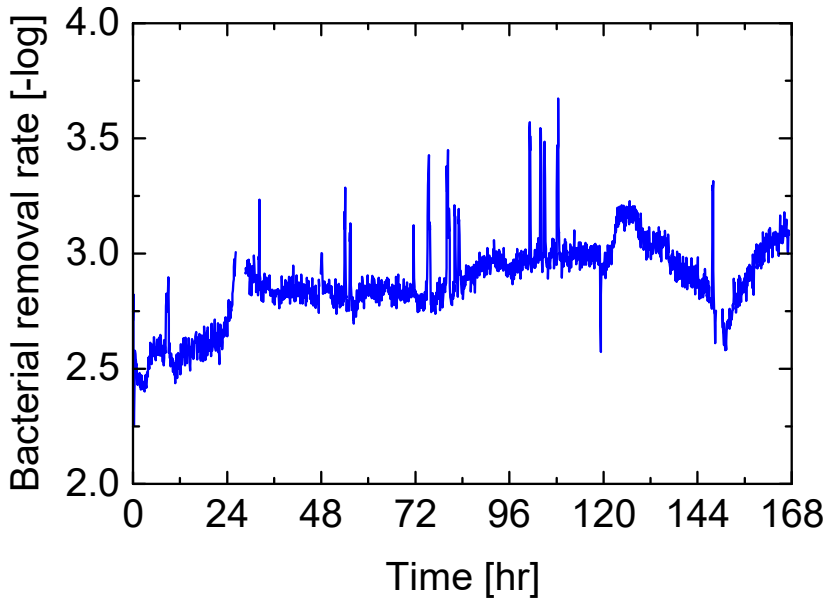

Fig. S4 – Variations in bacterial removal rates as determined by the online bacterial counts during RO treatment of MBR effluent at the pilot scale.