Online assessment of sand filter performance for bacterial removal in a full-scale drinking water treatment plant

Takahiro Fujioka,[1,*] Tetsuro Ueyama,[1,2] Fang Mingliang,[3] Menu Leddy,[4]

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

[4]*Essential Environmental Engineering Solutions, Huntington Beach, CA 92649, USA*

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

Abstract

Microbiological risks associated with drinking water can be minimized by providing enhanced integrity monitoring of bacterial removal by water treatment processes. This study aimed to evaluate the efficacy of real-time bacteriological counters for continuously assessing the performance of a full-scale sand filter to remove bacteria. Over the course of an 8-day evaluation, online counting of bacteria was successfully performed, providing continuous bacterial counts in the sand filter influent and effluent over approximate ranges from 17×10^4 to 94×10^4 and from 0.2×10^4 to 1.3×10^4 counts/mL, respectively. Periodic variations were observed with online bacterial counts in the sand filter influent because of the changes in the performance of flocculation and sedimentation processes. Overall, online removal rates of bacteria determined during the full-scale test were 95.2–99.3% (i.e., 1.3–2.2-log), indicating that online bacterial counting can continuously demonstrate over 1.3-log removal in the sand filter. Real-time bacteriological counting technology can be a useful tool for assessing variability and detecting bacterial breakthrough. It can be integrated with other online water quality measurements to evaluate underlying trends and the performance of sand filters for bacterial removal, which can enhance the safety of drinking water.

Keywords: bacterial count; online monitoring; sand filtration; drinking water.

1 INTRODUCTION

Management of microbiological risks in drinking water is crucial to ensure the safety of drinking water for public health protection (Villanueva et al., 2014). Microbiological risks in drinking water are typically minimized by achieving a sufficient reduction in pathogenic microorganisms through multiple barriers including drinking water treatment processes (Prest et al., 2016). In a conventional rapid sand filtration system, the filtration process plays a critical role in removing protozoa (i.e., *Cryptosporidium* and *Giardia*). Therefore, the United States Environmental Protection Agency (US EPA) has set the Surface Water Treatment Rules (SWTRs), which requires filtration systems to achieve 2-log removal of *Cryptosporidium*. Nonmicrobial surrogates such as turbidity and/or particle counts are generally used to detect and monitor for waterborne pathogens including *Cryptosporidium*, because it can be difficult to sample and analyze protozoa at a frequency that can continuously assess sand filter performance for pathogen removal. However, online process monitoring can sensitively and economically detect breakthrough of nonmicrobial surrogates through filtration, this can detect failures for pathogen breakthrough.

As a surrogate indicator of pathogenic contamination in drinking water, total coliform has been widely used as a traditional indicator for water quality. Therefore, the Total Coliform Rule by US EPA sets a drinking water standard (maximum contaminant level goal) for total coliforms at zero cell/mL. Depending on the concentration of bacteria and inorganic matter, bacteria can be primarily inactivated through post-chlorination. In addition to disinfection, removal of bacteria by sand filtration further enhances the final water quality. To date, online analytical methods have not been fully established with respect to continuous monitoring of sand filter performance for bacterial removal. For monitoring purposes, conventional counting of total culturable bacteria is time consuming and uneconomical. Thus, development of new online real

time screening methods for microbial contaminants is important to continuously ensure sand filter integrity (Van Nevel et al., 2017; Liu et al., 2018; Sherchan et al., 2018).

New technologies for microbial analysis in drinking water applications include flow cytometry (FCM) (Vital et al., 2012; Prest et al., 2014; Samendra et al., 2014; Ou et al., 2017). FCM uses simultaneous light-scattering and fluorescence measurements coupled with dye staining for microbial counting and analysis; thus, bacterial counting using FCM is highly useful to measure microbial water quality. It has been validated for detecting variations in both intact and damaged bacterial cells for drinking water treatment systems and for distribution network (Props et al., 2018). FCM analysis has recently been automated with robotics for staining cells (Hammes et al., 2012; Besmer et al., 2014); providing further capabilities for online monitoring of bacterial counts. However, excessive dye volumes required for staining is a challenge for continuously monitoring sand filter performance.

Another online bacterial counting technique that has recently emerged is a real-time bacteriological counting technology, which is also based on simultaneous light-scattering and autofluorescence measurements (Pepper and Snyder, 2016; Fujioka et al., 2018). This technology utilizes the combined autofluorescence emitted from riboflavin and nicotinamide adenine dinucleotide - hydrogen (NADH) in bacterial cells to identify them in water. By using intrinsic fluorescence, concentrations of bacteria in water can be monitored in real time without dyes for staining and detecting bacteria in water. Thus, online real time bacteriological counters may be more practical for physical treatment processes that provide continuous bacterial counts before and after treatment (Fujioka et al., 2019a; Fujioka et al., 2019b). This technology can be applied to sand filtration to monitor performance for bacterial removal and detect breakthrough of bacteria. Nevertheless, the effectiveness of this approach has not been assessed in drinking water applications.

This study aimed to assess the efficacy of real-time and online bacteriological counting technology for continuously monitoring full-scale sand filter performance for bacterial attenuation and removal. Bacterial counts before and after (i.e., influent and effluent) were continuously measured to demonstrate the ability of online bacterial counting for tracking variations in bacterial removal by sand filter to assess the integrity of the full scale sand filter. The ultimate aim of this study was to monitor the sand filter and ascertain its performance through online counting of bacteria in the sand filter influent and effluent. Ultimately, online monitoring has the potential of enhancing the safety of drinking water.

2 MATERIALS AND METHODS

2.1 Drinking water treatment system

This full-scale study was conducted at a drinking water treatment plant in Sasebo (Nagasaki, Japan). This plant is comprised a water intake basin, two rapid mixing basins, four flocculation and sedimentation basins equipped with inclined plate separators, and six rapid sand filtration basins **(Fig. 1)**. Drinking water sources to the plant include both river and lake waters, which are mixed at the water intake basin. Size of each flocculation basin and sedimentation basin is 9.0 m (L) × 10.2 m (W) × 3.0 m (D) (capacity = 275.4 m^3) and 12.0 m (L) × 10.4 m (W) × 3.0 m (D) (capacity = 374.4 m^3), respectively. Poly-aluminium chloride (PAC) at an Al concentration of 10.3 w/w% was supplied by Central Glass Co. (Tokyo, Japan). Each sand filter comprises dual filter media: 0.3 m depth of anthracite (effective size = 1.2 mm, and uniformity coefficient = 1.4) and 0.35 m depth of fine sand (effective size = 0.6 mm, and uniformity coefficient = 1.4), which are supported on four gravel layers. Area of each filtration basin is 5.6 m (L) × 9.2 m (W), which is equal to the surface area of 51.4 m^2.

[Fig. 1]

2.2 *Analytical methods*

Real-time bacteriological counters (IMD-W^{TM}, Azbil Co., Tokyo, Japan) were used to monitor bacterial counts in both the influent and effluent of the sand filter. To count the number of bacterial particles, the analyzer detects the intensity of two different lights for particle counting and bacterial autofluorescence in response to the excitation (Ex) light (wavelength = 405 nm). The two lights are comprised of (a) scattered light for counting the number and size of particles in water and (b) two autofluorescence emission (Em) lights (wavelength = 410–450 and 490–530 nm) emitted from riboflavin and NADH in bacteria (and protozoa that is larger than bacteria). The analyzer has the capacity of detecting particles of >0.3 μm, and requires a sample flow rate of 10 mL/min and a water pressure of >70 kPa.

Bacterial counting using flow cytometry, epifluorescence microscopy, and plate count was performed using manually collected samples. Flow cytometric bacterial counts were measured using a flow cytometer (BD Accuri® C6, BD Biosciences, San Jose, CA, USA). The analytical instrument was set to irradiate the excitation light (wavelength = 488 nm) and to detect emission light through an optical filter (533/30 nm). SYBR Green I nucleic acid gel stain, which is generally used with flow cytometry measurements to count both dead and alive bacteria cells in natural water (Prest et al., 2016), was used for staining bacteria at 1% concentration with 20 min incubation time.

Epifluorescent bacterial counts were measured using a fluorescence microscope (Rapisco, Shibasaki, Inc., Chichibu, Japan). Samples were first diluted using pure water. Thereafter, 1 mL of each sample was filtered using a track-etched polycarbonate MF membrane with 0.2 μm pore size (Meric, Tokyo, Japan). Total number of both viable and nonviable bacteria (total direct bacterial counts) was measured with 4’-6-diamidino-2-phenylindole (DAPI) dye solution (Thermo Fisher Scientific, Waltham, MA, USA). Compared to SYBR GREEN

staining, DAPI will generally provide lower bacterial counts; however both methods show a high correlation (Shibata et al., 2007). Alive direct bacterial counts were calculated by subtracting of the dead bacterial cell counts measured using 3,6-Bis(dimethylamino)acridine hydrochloride solution (Dojindo Laboratories, Kumamoto, Japan) from total bacterial counts.

Viable bacterial counts were also measured using standard plate count agar (PCA) method. Each sample (1 mL) was added to 15 mL sterile standard plate count agar medium (E-KB07, Eiken Chemical, Tokyo, Japan) at a temperature of 45°C. They were mixed, transferred into sterile Petri dishes, and incubated at 20°C for 22–26 hrs, as per the Drinking Water Quality Standards in Japan. Bacterial counts using PCA methods were expressed as colony-forming unit (CFU). Heterotrophic plate count (HPC) method was also used to determine the number of total viable bacteria in water that use organic carbon. The HPC method used R2A medium (Kanto Chemical, Tokyo, Japan); plates were incubated at 20–25 °C and counted after seven days. The other conditions were same as the standard PCA method.

2.3 Experimental protocols

Sand filter #3 was evaluated for bacterial removal. Effluent from the sedimentation basin #2 was collected as the sand filter influent, which was denoted as "filter influent" throughout this study **(Fig. 1)**. Filtrate of the sand filter #3 was referred as "filter effluent". During the tests, backwashing of the sand filter #3 was conducted for one hour at 18–19, 90–91, and 162–163 hrs. It is important to note that the frequency of backwashing at the full-scale plant during this study period was once every three days, however, throughout the year it can vary depending on the increase in turbidity or the head loss of the filters. To reduce the background interference from dissolved organics (humic acid-like organic matter) that are not counted but can mask autofluorescence of bacteria, an online sample dilution method that was previously reported in literature (Fujioka et al., 2018) was applied. Before analysis, the filter influent and effluent

underwent 50- and 3-fold dilution using the pure water, respectively **(Fig. S1)**. The pure water was prepared by treating tap water using a reverse osmosis (RO) membrane system followed by a microfiltration (MF) filter. Online bacterial counting of the effluent continued over the course of 184 hrs so that the variations in the bacterial concentration in the raw water matrix and the filter performance were examined for a sufficient period of time. It is noted that online analysis of the filter influent was not performed for the first 17 h. Grab samples for manual water quality analysis were collected once on weekdays (i.e., 17, 41, 65, 89, and 184 hr). Residual chlorine in the samples (0.3–0.6 mg-Cl_2/L in the filter influent) was quenched with sodium thiosulphate in the sample bottles. Bacterial log removal was calculated as follows:

$$Bacterial\ log\ removal = \log_{10}\frac{A}{B}$$

A and *B* are values for bacterial counts (counts/mL) in the filter influent and effluent.

3 RESULTS AND DISCUSSION

3.1 Online data

Over the course of an 8-day test, variations in the flow rate of raw intake water (approximately between 800 and 1,200 m^3/h) occurred because of mixing of lake water from midnight to 2–3 pm daily **(Fig. 2a)**. The river water was consistently supplied at approximately 800 m^3/h daily, whereas the lake water was intermittently supplied at approximately 370–400 m^3/h to meet the increased water demands. According to the intake flow rate, retention time at each flocculation and sedimentation basin at fixed capacity varied from 55–83 and 75–112 min, respectively, which was expected to influence their treatment performance. Coagulation with PAC was performed at a constant coagulant dose of 6.3–8.8 mg-Al/L **(Fig. S2a)**. After the sedimentation process, the effluent underwent intermediate chlorination with a constant hydrochloric acid dose of 1.5-1.7 mg-Cl_2/L **(Fig. S2a)**. It is noted that the intermediate chlorination process is

mainly designed to minimize the growth of algae and bacteria in the sand filter basin. The flow rate at sand filter #3 ranged from 1.7 to 4.8 m^3/m^2h according to the changes in intake flow rate and backwashing of the other filtration basins **(Fig. S2b)**. During the testing period, turbidity before the sand filter varied in the range of 0.3–1.5 mg/L almost every 12 hrs **(Fig. S3a)**. Accordingly, turbidity in the filter effluent varied from 0.01 to 0.06 mg/L **(Fig. S3b)**.

[Fig. 2]

During the evaluation, online bacterial counts in the filter influent considerably varied from 17×10^4 to 94×10^4 counts/mL **(Fig. 2b)**. This occurred at the same frequency as the variation in intake flow rate **(Fig. 2)**. The variable intake flow rate can change the retention time of the water in the flocculation and sedimentation basins, which is directly linked with the efficiency of floc formation and sedimentation and can vary the bacterial counts in the effluent (i.e., filter influent). Online bacterial counts in filter effluent were low, ranging from 0.2×10^4–1.3×10^4 counts/mL; this was approximately two orders of magnitude lower than those in the filter influent **(Fig. 2b)**. In general, online bacterial counts in the filter influent showed an underlying downward trend, whereas those in the filter effluent did not follow the trend. In response to the periodical changes in intake flow rate, online bacterial counts in both influent and effluent varied periodically. Additionally, a sudden increase of bacterial counts in the filter effluent was observed after backwashing of sand filter #3 at 18–19, 90–91, and 162–163 hrs. As previously reported, a similar increase in residual turbidity in the filter effluent after backwashing can be found in drinking water applications (Ahmad et al., 1998). Following backwashing, the breakthrough of bacteria in the sand filters can occur due to direct pass through and release of bacteria retained on sand particles. Bacteria, which were not removed by backwashing (i.e., bacteria retained between sand particles), are more likely to pass through the sand filters, causing the sudden increase in bacterial counts soon after backwashing. Overall, the results

here showed that online bacterial monitoring can detect variations and track the overall trends in bacterial counts in the filter influent and effluent. This indicates that online bacterial monitoring can be used as a sensitive tool for rapidly detecting bacteria-related issues such as spikes and breakthrough.

3.2 Manual bacterial counts

To assess the variance among bacterial counting techniques and identify the level of microbial contaminant in the filter effluent, microbial water quality analysis with additional methods (i.e., epifluorescence microscopy, flow cytometry, PCA, and HPC) was conducted using manually collected samples at five different sampling events. In the filter influent, the epifluorescent counts that measure the total bacterial counts (27×10^4–82×10^4 counts/mL) and alive bacterial counts (8×10^4–26×10^4 counts/mL) were similar to the online bacterial counts recorded at the time of sample collection (43×10^4–89×10^4 counts/mL) **(Table S1)**. However, a clear correlation between the two parameters was not established **(Fig. 3a)**. Similarly, flow cytometric bacterial counts in the filter influent (7×10^4–16×10^4 counts/mL) showed no correlation with the online bacterial counts. Conventional bacterial counting with the PCA and HPC methods showed lower bacterial counts (22–77 and 650–1,700 CFU/mL, respectively) than the online bacterial counts in the filter influent. The difference in counts between the methods is typically found in surface water; the total bacterial community by HPC can be down to 0.001–8.3% of total direct bacterial counts (Van Nevel et al., 2017). In the filter effluent, the online bacterial counts (0.6×10^4–1.3×10^4 counts/mL) were greatly reduced than the epifluorescent counts for total bacterial (6.3×10^4–13.6×10^4 counts/mL) and alive bacteria (1.5×10^4–4.5×10^4 counts/mL) but similar to flow cytometric bacterial counts (0.2×10^4–1.0×10^4 counts/mL) **(Table S1)**. Among the results, online bacterial counts showed a relatively high correlation with total direct bacterial counts (r = 0.93) and flow cytometric bacterial counts (r

= 0.85) **(Fig. 3b)**. Results showed that online bacterial counts in the filter influent are generally not well correlated with those by other conventional and emerging methods. This is likely due to the fundamental difference in the principle of bacterial counting among these techniques. Real-time bacteriological counter identifies most bacteria (>0.2 μm) but relies on the autofluorescence intensity of bacteria. It is not fully established whether all alive bacterial cells are counted, because autofluorescence of bacteria can be varied for various reasons such as cell viability. In this study, alive bacterial counts did not show a significant correlation with online bacterial counts for both filter influent and effluent either. Therefore, for future studies, it is important to identify what state of bacteria is actually counted by online bacterial counter and whether there is an algorithm (e.g., the ratio between two autofluorescent metabolites) to deconvolute the alive and dead cells.

[Fig. 3]

3.3 Variations in bacterial removal rate

Online bacterial counts in both the filter influent and effluent throughout the full-scale test provided bacterial removal rates over the ranges from 95.2% to 99.3%, which corresponds to removal rates of 1.3–2.2-log **(Fig. 4)**. Bacterial removal rates showed an underlying downward trend during filtration. Daily fluctuations in log removal rate appeared to be associated with the fluctuation in online bacterial counts in the filter influent, however, this was not considerable. More importantly, despite the remarkable increase in bacterial counts in the filter effluent after each backwashing event, log removal rate dropped only slightly from 2.16- to 1.95-log, from 1.93- to 1.68-log, and from 1.68- to 1.56-log for the backwashing at 18, 90, and 162 hr, respectively; indicating backwashing with sufficient drain prior to restart of sand filtration ensures the filter water quality. Further data analysis indicated that there was a 90% probability that the bacterial log removal rates range between 1.5–2.0-log **(Fig. 5)**. The performance

distribution curve was relatively broad with the average and standard deviation of 1.7-log and 0.2-log, respectively. These findings demonstrate that real-time bacteriological counting technology can be useful for monitoring the sand filter process and its ability to remove bacterial contaminants. The online monitoring technology has the potential of continuously demonstrating approximately a 1.5-log removal of bacteria by the sand filtration process. Additionally, the results demonstrate that continuous monitoring of bacterial counts before and after sand filtration provides a profile of the variation in the counts and the underlying trends in bacterial removal by the sand filtration process, which cannot be determined otherwise.

[Fig. 4]

[Fig. 5]

The cause and effect of the variations in bacterial removal rate during the full-scale performance tests was beyond the scope of this study. The size and shape of bacteria and biofilm formation on the sands can be an important properties for determining the extent of their removal during the filtration process (Kristian Stevik et al., 2004; Bai et al., 2016); bacteria larger than 1 μm size are more likely to be retained by the sand filtration process (Gannon et al., 1991). Additionally, the community composition of bacterial species can vary considerably daily and seasonally. In addition, microbiomes in drinking water are very complex with up to forty eight (48) phyla including predominant *Protebacteria* (Proctor and Hammes, 2015). Next-generation DNA sequencing of 16S rRNA (Liu et al., 2018) can help identify which bacterial species are passing through the sand filter.

3.4 Full-scale implications

Real-time bacteriological counting technology can provide online measurements of bacterial counts, which can be integrated with conventional online water quality measurements (i.e.,

turbidity) to enhance the safety of drinking water. Collection of online measurements during individual filter operation establishes operational baselines for bacterial counts in the filter influent and effluent, and provides a more accurate assessment of bacterial removal rates. The range and variation in bacterial counts during the earlier stages of the treatment process can help to detect spikes and unacceptable breakthrough of bacteria in a drinking water treatment systems during minimal incremental failure. This information can act as an early warning that necessitates to implement countermeasures such as increasing coagulation and/or disinfection. Determining the baseline level of bacterial counts at each treatment process provides greater information to investigate the cause of unexpected events in microbial water quality by analyzing historical data. In addition, baseline bacterial counts and removal rates can help to detect failures and changes in the separation performance and integrity of sand filter process. Compared to turbidity as a classical surrogate indicator for bacterial contamination, bacterial count is more relevant to microbial quality. Thus, in addition to conventional turbidity monitoring, online bacterial monitoring for bacterial removal can enhance the safety of drinking water.

Bacterial removal by sand filter has the potential to be used as a surrogate for controlling sand filter process for the removal of protozoa (e.g. *Cryptosporidium* and *Giardia*). The removal of bacteria and protozoa by sand filtration is dependent upon size exclusion mechanisms along with other mechanisms such as adsorption and sedimentation. Thus, the removal of protozoa (>1 μm in size) can be expected to be greater than bacteria (>0.2 μm in size) because of size. Because the performance of full-scale media filtration processes for bacterial removal can be variable depending on the conditions of the filter (e.g., clogging level, backwashing, and effective media size), the enhanced integrity monitoring for protozoa removal at each filter basis can improve the safety of drinking water. Further sand filtration studies are needed to

verify the use of bacterial removal as a surrogate of protozoa removal through long-term full-scale evaluations.

4 Conclusions

This study for the first time assessed the efficacy of real-time bacteriological counters for continuously and economically measuring bacterial counts in the sand filter influent and effluent at a full-scale drinking water treatment plant. Bacterial counts in the sand filter influent and effluent were continuously determined over approximate ranges from 17×10^4 to 94×10^4 and from 0.2×10^4 to 1.3×10^4 counts/mL, respectively. Online bacterial counts in the filter influent and effluent varied in response to changes in flow rate and backwashing. Online removal rates of bacteria determined during the full-scale test were 1.3-2.2 log (i.e., 95.2–99.3%). This indicates that online bacterial counting can continuously demonstrate over 1.3-log removal in sand filter performance. This also suggests that online bacterial monitoring can be a useful tool to ascertain log removal rates by sand filtration process. Monitoring the bacterial removal rates can be integrated with conventional online water quality measurements in sand filter effluent (e.g., turbidity and particle counts) to enhance the safety of drinking water.

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6 REFERENCES

Ahmad, R., Amirtharajah, A., Al-Shawwa, A., Huck, P.M., 1998. Effects of backwashing on biological filters. Journal - American Water Works Association 90, 62-73.

303 Bai, H., Cochet, N., Pauss, A., Lamy, E., 2016. Bacteria cell properties and grain size impact
304 on bacteria transport and deposition in porous media. Colloids Surf. B. Biointerfaces
305 139, 148-155.

306 Besmer, M.D., Weissbrodt, D.G., Kratochvil, B.E., Sigrist, J.A., Weyland, M.S., Hammes, F.,
307 2014. The feasibility of automated online flow cytometry for in-situ monitoring of
308 microbial dynamics in aquatic ecosystems. Frontiers in Microbiology 5, 265.

309 Fujioka, T., Hoang, A.T., Aizawa, H., Ashiba, H., Fujimaki, M., Leddy, M., 2018. Real-Time
310 Online Monitoring for Assessing Removal of Bacteria by Reverse Osmosis. Environ.
311 Sci. Technol. Letters 5, 389-393.

312 Fujioka, T., Hoang, A.T., Ueyama, T., Nghiem, L.D., 2019a. Integrity of reverse osmosis
313 membrane for removing bacteria: new insight into bacterial passage. Environ. Sci.:
314 Water Res. Technol. 5, 239-245.

315 Fujioka, T., Makabe, R., Mori, N., Snyder, S.A., Leddy, M., 2019b. Assessment of online
316 bacterial particle counts for monitoring the performance of reverse osmosis membrane
317 process in potable reuse. Sci. Total Environ. (In Press,
318 10.1016/j.scitotenv.2019.02.339).

319 Gannon, J.T., Manilal, V.B., Alexander, M., 1991. Relationship between Cell Surface
320 Properties and Transport of Bacteria through Soil. Appl. Environ. Microbiol. 57, 190-
321 193.

322 Hammes, F., Broger, T., Weilenmann, H.-U., Vital, M., Helbing, J., Bosshart, U., Huber, P.,
323 Peter Odermatt, R., Sonnleitner, B., 2012. Development and laboratory-scale testing of
324 a fully automated online flow cytometer for drinking water analysis. Cytometry Part A
325 81A, 508-516.

326 Kristian Stevik, T., Kari, A., Ausland, G., Fredrik Hanssen, J., 2004. Retention and removal of
327 pathogenic bacteria in wastewater percolating through porous media: a review. Water
328 Res. 38, 1355-1367.

329 Liu, G., Zhang, Y., van der Mark, E., Magic-Knezev, A., Pinto, A., van den Bogert, B., Liu,
330 W., van der Meer, W., Medema, G., 2018. Assessing the origin of bacteria in tap water
331 and distribution system in an unchlorinated drinking water system by SourceTracker
332 using microbial community fingerprints. Water Res. 138, 86-96.

333 Ou, F., McGoverin, C., Swift, S., Vanholsbeeck, F., 2017. Absolute bacterial cell enumeration
334 using flow cytometry. J. Appl. Microbiol. 123, 464-477.

335 Pepper, I.L., Snyder, S.A., 2016. Monitoring for reliability and process control of potable reuse
336 applications. Water Environment & Reuse Foundation and IWA Publishing,
337 Alexandria, VA.

338 Prest, E.I., El-Chakhtoura, J., Hammes, F., Saikaly, P.E., van Loosdrecht, M.C.M.,
339 Vrouwenvelder, J.S., 2014. Combining flow cytometry and 16S rRNA gene
340 pyrosequencing: A promising approach for drinking water monitoring and
341 characterization. Water Res. 63, 179-189.

342 Prest, E.I., Hammes, F., van Loosdrecht, M.C.M., Vrouwenvelder, J.S., 2016. Biological
343 Stability of Drinking Water: Controlling Factors, Methods, and Challenges. Frontiers
344 in Microbiology 7, 1-24.

345 Proctor, C.R., Hammes, F., 2015. Drinking water microbiology—from measurement to
346 management. Curr. Opin. Biotechnol. 33, 87-94.

347 Props, R., Rubbens, P., Besmer, M., Buysschaert, B., Sigrist, J., Weilenmann, H., Waegeman,
348 W., Boon, N., Hammes, F., 2018. Detection of microbial disturbances in a drinking
349 water microbial community through continuous acquisition and advanced analysis of
350 flow cytometry data. Water Res. 145, 73-82.

351 Samendra, P.S., Masaaki, K., Charles, P.G., Ian, L.P., 2014. Rapid Detection Technologies for
352 Monitoring Microorganisms in Water. Biosensors Journal 3, 109.

353 Sherchan, S., Miles, S., Ikner, L., Yu, H.-W., Snyder, S.A., Pepper, I.L., 2018. Near Real-Time
354 Detection of E. coli in Reclaimed Water Sensors 18, 2303.

355 Shibata, A., Imai, A.K.I., Hara, S., Kikuchi, T., Toda, T., Taguchi, S., 2007. Quantitative
356 difference in bacterial abundance determined with each protocol for SYBR Green I and
357 4'6-diamidino-2-phenylindole (DAPI) methods. Plankton and Benthos Research 2, 63-
358 66.

359 Van Nevel, S., Koetzsch, S., Proctor, C.R., Besmer, M.D., Prest, E.I., Vrouwenvelder, J.S.,
360 Knezev, A., Boon, N., Hammes, F., 2017. Flow cytometric bacterial cell counts
361 challenge conventional heterotrophic plate counts for routine microbiological drinking
362 water monitoring. Water Res. 113, 191-206.

363 Villanueva, C.M., Kogevinas, M., Cordier, S., Templeton, M.R., Vermeulen, R., Nuckols, J.R.,
364 Nieuwenhuijsen, M.J., Levallois, P., 2014. Assessing exposure and health
365 consequences of chemicals in drinking water: current state of knowledge and research
366 needs. Environ. Health Perspect. 122, 213-221.

367 Vital, M., Dignum, M., Magic-Knezev, A., Ross, P., Rietveld, L., Hammes, F., 2012. Flow
368 cytometry and adenosine tri-phosphate analysis: Alternative possibilities to evaluate
369 major bacteriological changes in drinking water treatment and distribution systems.
370 Water Res. 46, 4665-4676.

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FIGURES

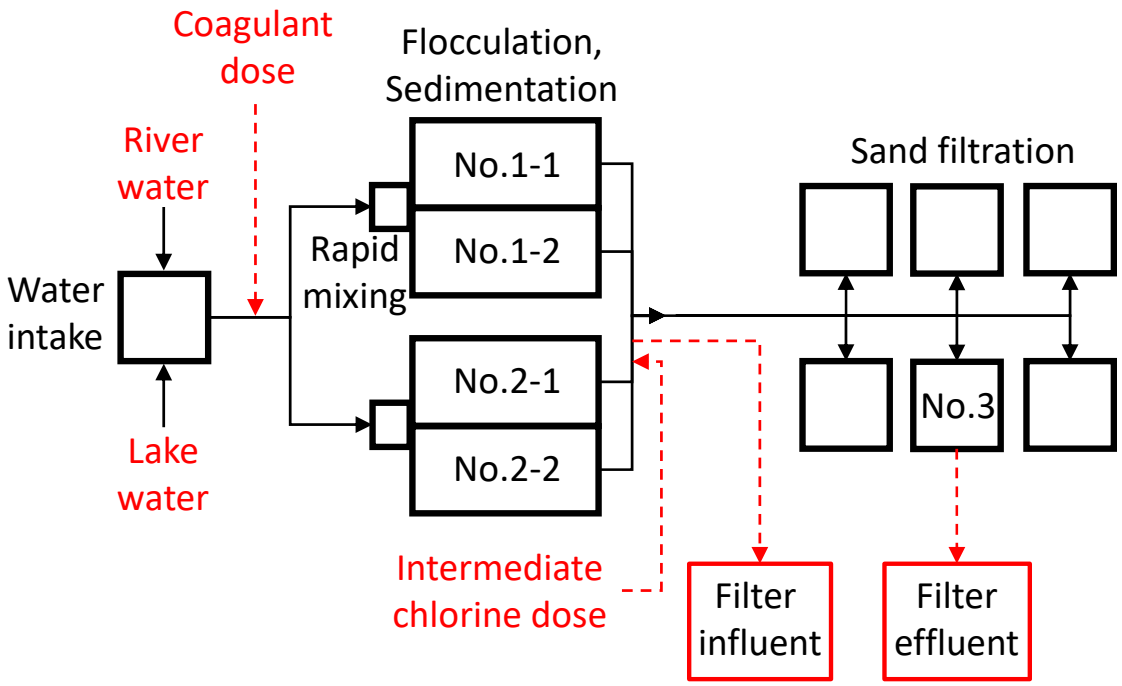
Fig. 1 – Schematic flow diagram of the drinking water treatment plant.

Fig. 2 – (a) Flow rate of raw water intake and (b) online bacterial counts in the filter influent and effluent (Data recorded for 5 min was averaged and plotted. Backwashing was conducted for one hour at 18–19, 90–91, and 162–163 hrs).

Fig. 3 – Bacterial counts determined by epifluorescent microscopy and flow cytometry as a function of online bacterial counts in the (a) filter influent and (b) filter effluent. Error bars show standard deviations for analytical triplicates.

Fig. 4 – Bacterial removal rates as determined by online bacterial counters. Backwashing was conducted for one hour at 18–19, 90–91, and 162–163 hrs.

Fig. 5 – Process performance probability distribution of bacterial removal rates as determined by online bacterial counters (Average = 1.7-log, STDEV = 0.2-log).


Coagulant dose
Flocculation, Sedimentation
River water
No.1-1
Sand filtration
Rapid mixing
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Water intake
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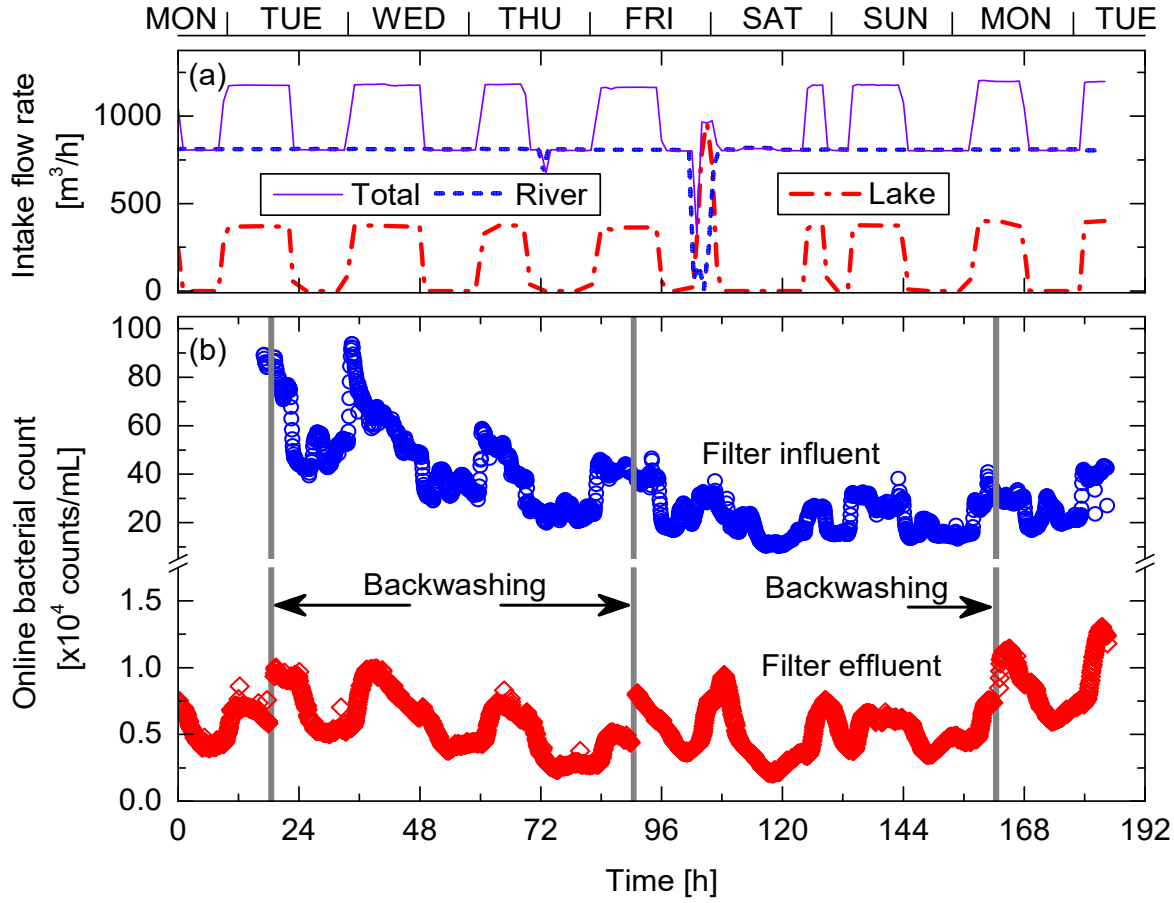
No.2-2
Lake water
Intermediate chlorine dose
Filter influent
Filter effluent

Fig. 1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100
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20
Filter influent
Backwashing
Backwashing
1.5
1.0
0.5
0.0
Filter effluent
0
24
48
72
96
120
144
168
192
Time [h]

Fig. 2

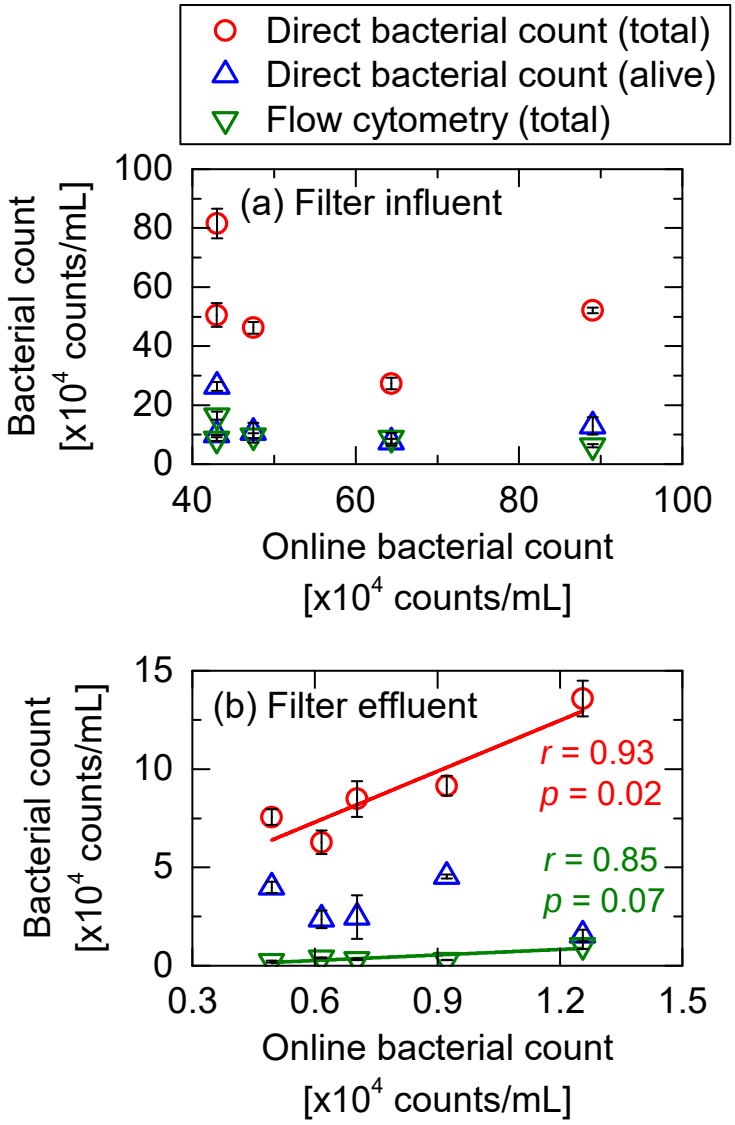
Direct bacterial count (total)
Direct bacterial count (alive)
Flow cytometry (total)
(a) Filter influent
Bacterial count
[x10^4 counts/mL]
Online bacterial count
[x10^4 counts/mL]
(b) Filter effluent
r = 0.93
p = 0.02
r = 0.85
p = 0.07
Bacterial count
[x10^4 counts/mL]
Online bacterial count
[x10^4 counts/mL]

Fig. 3

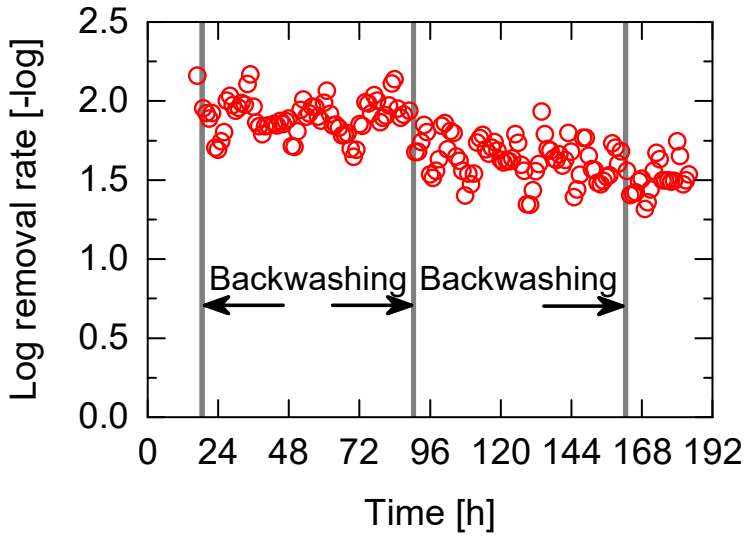
Log removal rate [-log]
2.5
2.0
1.5
1.0
0.5
0.0
Backwashing
Backwashing
0
24
48
72
96
120
144
168
192
Time [h]

Fig. 4

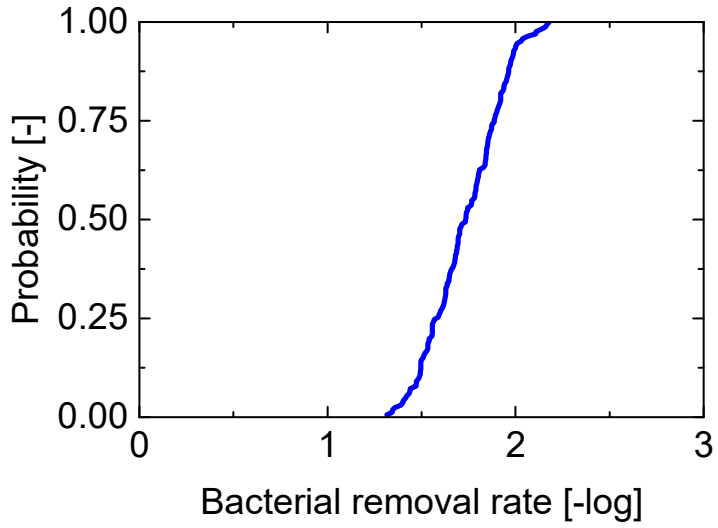
1.00
0.75
0.50
0.25
0.00
Probability [-]
0
1
2
3
Bacterial removal rate [-log]

Fig. 5

Supporting Information

Online assessment of sand filter performance for bacterial removal in a full-scale drinking water treatment plant

Takahiro Fujioka,[1,*] Tetsuro Ueyama,[1,2] Fang Mingliang,[3] Menu Leddy,[4]

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

[4]*Essential Environmental Engineering Solutions, Huntington Beach, CA 92649, USA*

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

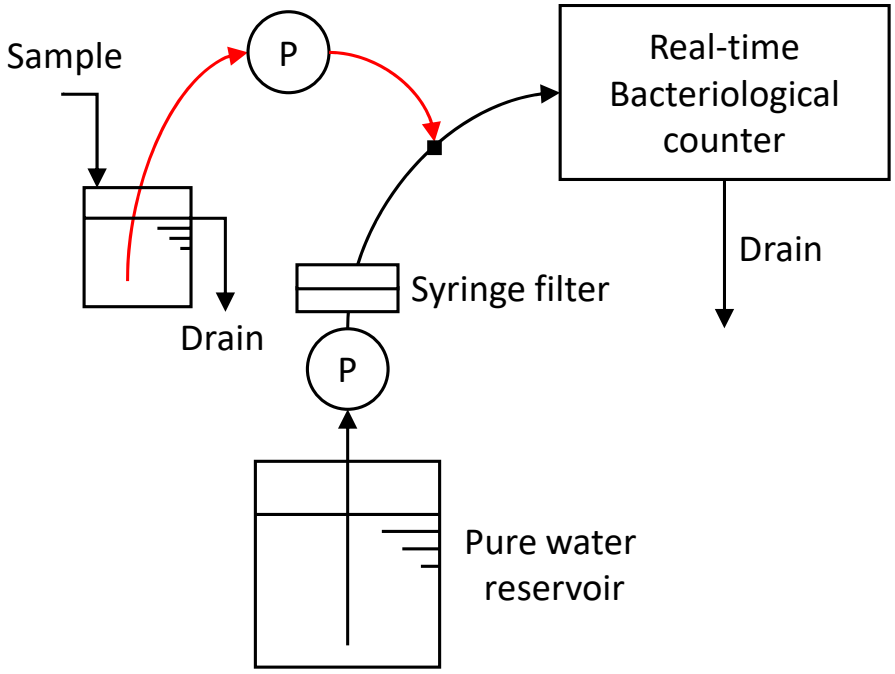

Fig. S1 – Schematic flow diagram of the dilution system for a real-time bacteriological counter.

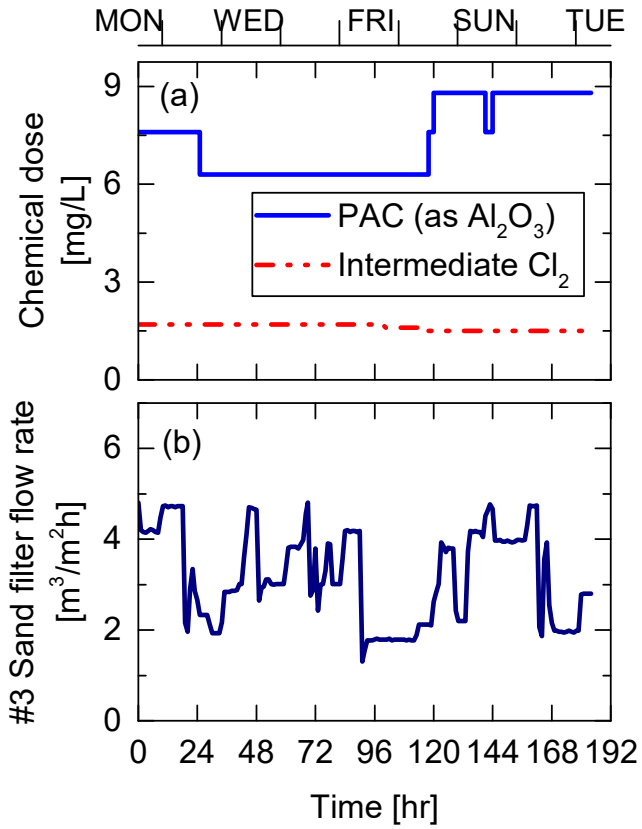

Fig. S2 – Water treatment system operating data: (a) chemical dose, and (b) flow rate of sand filter #3.

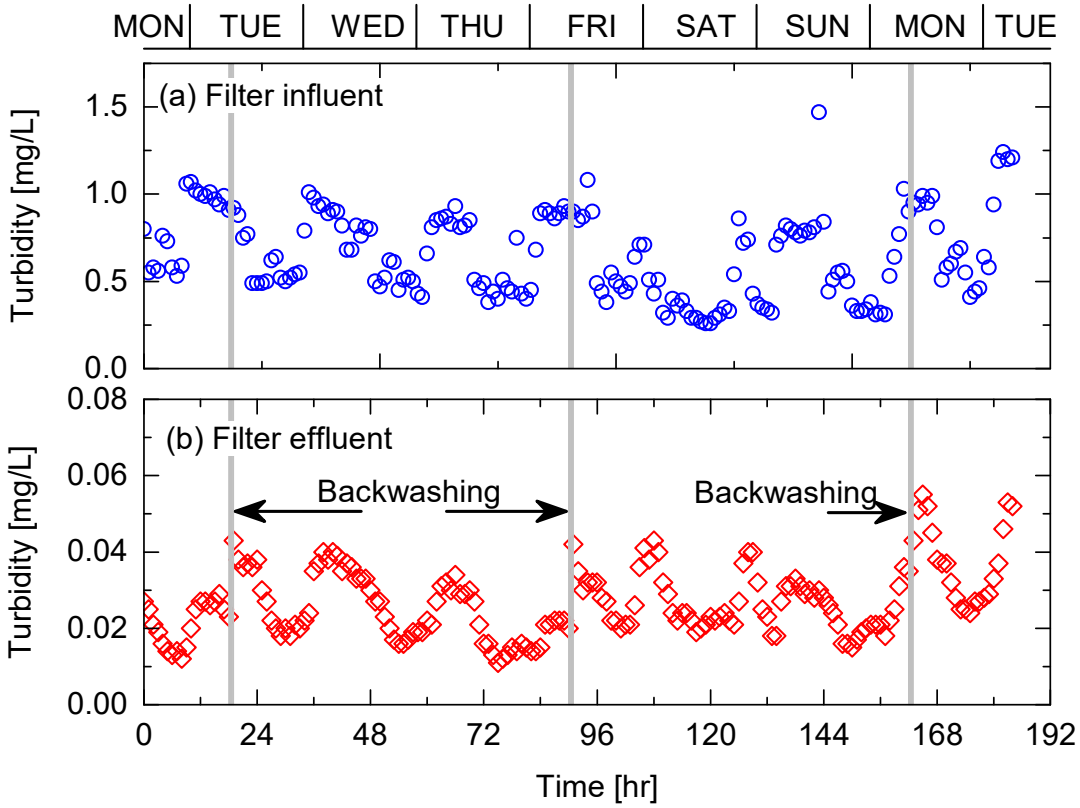

Fig. S3 – Online-monitored turbidity (plot every one hour) in the (a) filter influent and (b) filter effluent. Turbidity is expressed in units of mg/L, as per the turbidity standard in Japan. Backwashing was conducted for one hour at 18–19, 90–91, and 162–163 hrs. Turbidity in the filter influent and effluent was monitored online using an online surface scattering light turbidity analyzer (AN450A, Hitachi High-Tech Solutions Co., Tokyo, Japan) and an online laser light turbidity analyzer (AN455A, Hitachi High-Tech Solutions Co., Tokyo, Japan), respectively.

Table S1 – Manual grab sampling data during the full-scale performance test.

Time [h]		17	41	65	89	184
Filter influent						
Total direct bacterial count (epi-fluorescence)	[$\times 10^5$ counts/mL]	5.2 ±0.1	2.7 ±0.2	4.6 ±0.2	5.0 ±0.4	8.2 ±0.5
Alive direct bacterial count (epi-fluorescence)	[$\times 10^5$ counts/mL]	1.3	0.8	1.1	1.0	2.6
Flow cytometry	[$\times 10^5$ counts/mL]	0.66 ±0.05	0.88 ±0.19	0.97 ±0.08	0.85 ±0.06	1.64 ±0.14
PCA	[CFU/mL]	29	55	26	20	77
HPC	[CFU/mL]	N.A.	N.A.	1300	650	1700
Online bacterial count	[$\times 10^5$ counts/mL]	8.9	6.4	4.8	4.3	4.3
Filter effluent						
Total direct bacterial count (epi-fluorescence)	[$\times 10^4$ counts/mL]	6.3 ±0.6	9.2 ±0.5	8.5 ±0.9	7.6 ±0.4	13.6 ±0.9
Alive direct bacterial count (epi-fluorescence)	[$\times 10^4$ counts/mL]	2.4	4.5	2.5	4.0	1.5
Flow cytometry	[$\times 10^4$ counts/mL]	0.40 ±0.03	0.29 ±0.02	0.32 ±0.04	0.21 ±0.06	1.03 ±0.17
PCA	[CFU/mL]	0	0	0	0	0
HPC	[CFU/mL]	N.A.	N.A.	0	0	0
Online bacterial count	[$\times 10^4$ counts/mL]	0.62	0.92	0.70	0.49	1.26