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Application of the CANARY event detection software for real-time performance monitoring of decentralized water reuse systems†

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Abstract

Real-time monitoring of water reuse systems ensures the production of high quality water to protect human health at the point-of-use. In this study, several online real-time sensors were utilized to monitor effluent from a wastewater fed laboratory-scale membrane bioreactor (MBR) under natural and simulated failure conditions. These simulated failures included adding reactor mixed liquor to emulate a membrane breach, and spiking MS2 bacteriophage into the reactor to create a high viral load, which might be observed during an outbreak. The CANARY event detection software was used to analyze sensor data and report changes in water quality that might be indicative of poor system behavior. During simulated failure conditions, CANARY reported 20 alarms, accurately detecting each failure. During natural operating conditions, 219 alarms were produced and 189 were attributed to known events (*e.g.*, system and sensor maintenance). The remaining alarms (23) during natural operating conditions were considered to have an unknown cause. However, 13 of those had signal deviations similar to known events, but could not be definitively linked to a source. The results of this study suggest that real-time monitoring in conjunction with CANARY analysis may be useful as an early warning system for monitoring the effluent of water reuse systems, and may help to quickly identify treatment malfunctions or other abnormal conditions.

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1. Introduction

The onsite reuse of treated wastewater has gained attention as an option to reduce the burden placed on potable water systems during periods of prolonged drought or increased demand.[1] Water reuse can be broadly divided into potable and non-potable applications, and treatment requirements vary depending upon the expected end use.[1] Due to expected human use and subsequent exposure to reclaimed water, there is a critical need to guarantee reuse water quality to ensure the protection of public health.[2] Membrane bioreactors (MBRs) are one technology being considered for reuse applications because they can be configured for both centralized and decentralized reuse systems due to their low footprint, ease of scalability, and high-quality effluent.[3] Once a decentralized water reuse system is set up, effective monitoring schemes are needed to ensure that treatment units are performing as intended to remove contaminants related to adverse health outcomes.

The greatest acute risk to human health in water reuse systems is generally recognized to arise from viral pathogens of enteric origin.[1,4,5] Due to the number and diversity of fecal pathogens that could be present in wastewaters, microbial presence after treatment processes is typically monitored using a fecal indicator organism such as *E. coli*, fecal streptococci, or bacteriophage.[6,7] The use of fecal indicator organisms has been assimilated into drinking and recreational water quality regulations worldwide,[8] but traditional culture-based methods typically take 24–48 hours to obtain results.[9] Molecular-based methods (*e.g.*, PCR) targeting fecal indicators can improve response time to a few hours, but results are not obtained in real-time and only indicate presence of fecal material, not pathogens.[10,11]

Long analysis times associated with microbial methods have prompted interest in real-time detection and monitoring of treated water quality, particularly in the drinking water utility sector. However, currently available technology does not directly detect biological contaminants in drinking water distribution systems in real-time.[10] The drinking water sector has focused on the use of physicochemical sensors for real-time monitoring of water quality, where changes in one or more parameters have been associated with decreased water quality.[10,12,13] Sensor monitoring may be part of an early warning system for identifying poor water quality so that system operators can rapidly respond to and limit or prevent adverse impacts to customers.

Real-time water quality sensors are available to measure parameters such as pH, oxidation–reduction potential (ORP), dissolved oxygen (DO), residual chlorine, free chlorine, ammonia, nitrate, turbidity, conductivity, and temperature. More advanced equipment can perform ultraviolet (UV) spectrophotometry, fluorescent spectroscopy, multiple-angle light scattering, detection of adenosine triphosphate, and flow cytometry. These sensors can describe water quality changes, and studies in the drinking water sector utilizing real-time sensors have concluded that no single parameter is capable of responding to all contaminants, which highlights the need to use a multi-parameter approach to improve contaminant detection.[12,14,15] As water management plans continue to adopt wastewater reuse strategies such as the one proposed at the San Francisco Public Utilities Commission, [16] there is a heightened need to evaluate real-time monitoring strategies in water reuse scenarios.

Zhao *et al.* (2012) recently demonstrated a "real-time water quality information acquisition system" to detect changes in water quality in both raw sewage entering an Australian wastewater treatment plant and in the treated wastewater leaving the plant.[17] They deployed a network of sensors measuring pH, DO, ORP, temperature, turbidity and conductivity. An event detection system (EDS) was also deployed that analyzed real-time sensor data to detect anomalous patterns in the noisy water quality data. The tested system performed well, detecting stormwater overflows, industrial dumping of chemicals, and equipment failures. Failures of the treatment process were also detected during some of the dumping events. Zhao *et al.*[17] did not address microbial or viral breakthrough of wastewater treatment.

EDSs have primarily been deployed to detect water quality problems in drinking water.[18] EDSs can be configured in a variety of ways based on available sensors, monitoring objectives, and required response time. The configuration process typically focuses on minimizing false alarms while maximizing true detections. Many EDSs use setpoints for each water quality parameter, above or below which an alarm triggers. Most EDSs also incorporate predictive algorithms that generate alarms when the difference between predicted and recorded values (typically called the residuals) deviates more than a specified amount.[19] In this way, EDSs always alarm if parameter values exceed setpoints, but they also alarm if significant changes occur within these setpoint values. For water systems that apply the Hazard Analysis Critical Control Point (HAACP) approach, setpoints are equivalent to "alert" levels, which warn operators before parameters exceed "critical control points."[20] Using an EDS, then, can provide additional information about anomalous water quality behavior that could indicate a contamination incident or other water quality problem.

CANARY is an EDS software[21] that utilizes built-in or user-programmed prediction algorithms and setpoints to identify deviations from historical sensor behavior. CANARY can be configured to respond to a change in any individual sensor, or analyze sensors as a group, looking for simultaneous changes. The built-in algorithms can be further controlled by parameters provided within the input file. CANARY was originally developed to enhance the detection of contamination events in drinking water systems; however, the software is general enough that it can analyze time series data from any source. Event detection tools like CANARY have been adopted by drinking water utilities around the world to help continuously monitor their water quality.[22] CANARY is a free software tool available on EPA's website and has been incorporated into several commercially available data analytics packages.[23]

The objective of this study was to evaluate the response of CANARY EDS to data from real-time physiochemical water quality sensors monitoring effluent from a bench-scale ultrafiltration (UF) MBR treating sewer-mined wastewater. Following stabilization of the MBR, data was collected from a series of physicochemical sensors that logged measurements of the treated effluent every 30 seconds over a 114 day period and then analyzed by CANARY. Once stable operation of the MBR and online sensors was established, the system was challenged in two ways to 1) bypass mixed liquor suspended solids (MLSS) directly into the effluent line to simulate a membrane failure; and 2) spike MS2 bacteriophage before treatment to simulate high viral loading. The goal was to test whether the sensors would respond (*i.e.,* deviate from previous behavior) to expected

changes in effluent quality and to test whether CANARY would produce an alarm as a result of this sensor response. In addition to real-time sensor monitoring, grab samples were analyzed for quantities of adenovirus, and somatic and male-specific coliphage throughout the entire experimental period to determine if the alarms generated by CANARY had any association with viral presence. To the authors' knowledge, this is the first application of CANARY for monitoring wastewater treatment.

2. Materials and methods

2.1 Laboratory-scale MBR

A laboratory-scale UF MBR was used to treat wastewater. The 18 L reactor, seeded with activated sludge from the aeration tank of a local wastewater treatment plant (WWTP), contained a single aerobic compartment with two Zenon ZW-1 hollow fiber membrane modules (0.04 μm nominal pore diameter). The MBR was stabilized for longer than three solid retention periods for this system (>60 days) prior to biological sampling and data collection for CANARY analysis. The MBR was aerated at a rate of 2.3 $m^3\ h^{-1}$ to air scour the membrane surface, maintain DO, and provide mixing. Fine bubble diffusers were present for additional DO and mixing demands. Flow was drawn through the membrane at a net rate of 50 mL min^{-1}. The MBR was operated in two operational modes to study fouling reduction. For the first stage of operation, two relaxation cycles were executed concurrently: a 4 minute cycle with a 30 second pause and a 3 hour cycle with a 7 minute pause. The flow pauses relaxed the membrane allowing the aeration to remove foulants from the membrane surface. The second stage of operation utilized a 30 second permeate backflush every 6 minutes. The backflush cycles temporarily reversed the effluent flow to remove pore obstructions as well as surface foulants. The reverse flux was the same as the forward flux resulting in the same instantaneous flowrate as the relaxation cycle operation (60 mL min^{-1}, or a permeate flux of 38 L $h^{-1}\ m^{-2}$ (LMH)).

The MBR was operated normally until the manufacturer's recommended transmembrane pressure (TMP) limit of 59.9 kPa (17.7 in Hg) was reached. At this TMP, the influent and effluent flows were programmed to automatically shut down until the membranes could be replaced with a cleaned set. Cleaning consisted of a thorough tap water rinse, followed by soaking in a 1000 ppm sodium hypochlorite (NaOCl) solution for 6 hours. Cleaned membranes were stored in tap water until needed.

The reactor was fed by wastewater collected from a 48″ diameter sewer main running beneath the west campus of the University of Cincinnati in Cincinnati, Ohio, USA. It was collected using a pumping system consisting of a draw pump, a lift pump, a large storage tank, and a settling tank. The draw pump fed the storage tank from the sewer main and the lift pump transported the wastewater up to the laboratory and into the settling tank. In the settling tank, the wastewater was allowed to separate, with the solids and grit settling to the bottom and the fats and oils floating to the surface. Wastewater was drawn from a port located just below the middle of the tank to feed the reactor. Upstream of the draw pump connection to the sewer main are several restaurants, dorms, classroom buildings, and office buildings as well as runoff collection drains. All of these sources were assumed to have contributed to the sewage stream, though the exact contributions of each are unknown and

most likely varied widely over time. Traditional wastewater characteristics were measured daily from 24 hour composite samples of the influent while the MLSS was measured biweekly. Analysis was conducted for COD using Hach Method 8000, NH_3-N using Standard Method 4500-NH_3 D (Orion 9512HPBNWP Ammonia Electrode), nitrate using Standard Method 4500-NO_3 B, TKN using Hach Method 8075, and Total and Volatile Suspended Solids (TSS/VSS) using Standard Method 2540 D/E.[24] The solids retention time and hydraulic retention time were controlled at 20 days and 6 hours, respectively. The pH of the mixed liquor was measured daily (Oakton WD-35801–00 pH Electrode) and buffered with a 0.15 M sodium carbonate solution to maintain a range of 7 to 8. Data recorded after the initial stabilization period was used for event detection.

2.2 Online monitoring sensors

MBR effluent quality was monitored with a range of online sensors (Fig. 1): colored dissolved organic matter (CDOM) probe (Turner Design, Sunnyvale, CA); CSIM11 pH probe, CS511-L DO probe, and CS547a conductivity and temperature probe (Campbell Scientific, Logan, UT); Real UV254 M3000 ultraviolet spectrometer (RealTech, Ontario, CA); FT660sc nephelometer (turbidity) with sc200 controller (Hach, Loveland, CO); and 5310 C TOC analyzer (GE, Trevose, PA). These particular sensors were chosen since they are relatively inexpensive, are widely used, require little maintenance and allow frequent measurement of data. All instrument signals were recorded every 30 seconds using a CR1000 datalogger (Campbell Scientific, Logan, UT). All sensors were maintained per manufacturer's specifications.

2.3 CANARY event detection software

For this study, Version 4.3.2 of the CANARY software[25] was used on a dual core 2.26 GHz computer with 4 GB RAM. The signals for conductivity, DO, turbidity, UV_{254}, TOC, and CDOM-specific fluorescence were analyzed. Temperature and pH sensors were collected but not included in the CANARY analysis because they were not expected to be indicative of events for this system. The TMP and flow sensors were not analyzed by CANARY to detect events but were used to correlate system operational events to CANARY alarms.

The linear prediction–correction filter (LPCF) algorithm was used by CANARY. LPCF produces alarms when at least one sensor signal has prolonged deviations from its baseline. The LPCF algorithm predicts the current value of a signal for each sensor based on a user-defined number of previous data points (the history window). A data point is considered an outlier when the normalized residual (calculated as the difference between the actual and predicted values for each signal) exceeds the user-defined outlier threshold multiplied by the signal's standard deviation. If a data point is considered an outlier, it is not used for predicting future signal values. A probability is calculated based on the number of outliers in the user-defined binomial event discriminator (BED) window. For this study, an "alarm" from CANARY occurred when the probability exceeded the user-defined threshold parameter.[21] "Events" were defined as any known system disruption such as bypassing of the MLSS, spiking of MS2, or system maintenance performed by the operator. Using these definitions and system settings, a single event (*e.g.*, system maintenance) could generate more than one CANARY alarm. Alarms that coincided with events were considered alarms

with known causes, and those that did not correspond to an event were considered unknown alarms.

CANARY parameters may be adjusted to suit an application or desired monitoring objective. Using effluent sensor data, CANARY was configured following the parameter optimization procedure outlined in USEPA (2014).[26] Natural (discussed further in section 2.5) and simulated MBR failure (discussed further in sections 2.6 and 2.7) conditions were performed to parameterize CANARY to establish sensor profiles during baseline and known poor water quality events. The optimal configuration parameters selected were: history window = 800, outlier threshold = 1.1, BED window = 30, and event threshold = 1. Alarm durations were limited to 500 timesteps (250 minutes) using the event threshold parameter. The CANARY input file is included in the ESI.†

2.4 Microbial targets

2.4.1 Wastewater influent and mixed liquor suspended solids samples.—

The microbial quality of the influent and MLSS was determined by measuring adenovirus and somatic and male-specific coliphage. Standard Method 9224 Detection of Coliphages B/C[24] was used to analyze for somatic and male-specific coliphage samples.

For adenovirus analysis, 10 mL samples of the influent and MLSS were collected for genomic DNA extraction, followed by qPCR. Adenovirus genomic DNA was extracted using the QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA) following the manufacturer's instructions with slight modification. Buffer AVL (Qiagen) was used for lysis of viral capsids instead of Buffer AE and the use of protease was omitted. To elute genomic DNA from the QIAamp maxi columns, 1 mL of Buffer EB was applied and, following centrifugation, the entire eluate was reloaded onto the column for a second elution. To quantify the number of adenovirus genomes in DNA extracts, qPCR was used as described by Brinkman *et al.*[27] The primers and probes targeted the hexon gene of adenovirus species A–F as described by Jothikumar *et al.*[28] with slight modification; guanosine triphosphate replaced the inosine triphosphate at the third base position of the reverse primer (forward primer: 5′-GGACGCCTCGGAGTACCTGAG-3′; reverse primer: 5′-ACGGTGGGGTTTCTGAACTTGTT-3′; probe: 6FAMCTGGTGCAGTTCGCCCGTGCCA-BHQ1). Positive and negative controls consisting of adenovirus (OD 260 Inc., Boise, ID) and 10 mM Tris HCL, pH 8.5 were run in replicate on each qPCR plate. Adenovirus quantities in each PCR reaction were determined using most probable number (MPN) analysis of samples run at three dilutions (undiluted, 1 : 5, and 1 : 25) with 5 replicate reactions at each dilution as described in EPA Method 1615,[29] where the number of dilutions was set at 3, the number of tubes per dilution was set at 5 and the inoculum volume was set at 1. Quantities were then back calculated to the original 10 ml sample and normalized per mL of original sample.

2.4.2 MBR effluent samples.—

Due to expected low levels of target viruses in the effluent, the effluent stream was concentrated by adding a Rexeed® 25S hollow fiber

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ultrafilter (Dial Medical Supply, Chester Springs, PA) in line with the MBR effluent. The ultrafilter collected microbes in the effluent line continuously for up to 24 hours (72 L). After removal from the effluent line, the ultrafilter was eluted using 400 mL of a solution containing 0.01% (m/v) sodium polyphosphate (Sigma-Aldrich), 0.01% (v/v) Tween-80 (Sigma-Aldrich), and 0.001% (v/v) Y-30 Antifoam (Sigma-Aldrich). The solution was passed through the ultrafilter using a MasterFlex L/S peristaltic pump (Cole Parmer) set at 300 RPM (approximately 840 mL per minute) for 1 minute. The direction of the flow was reversed and elution solution was recirculated through the filter in the opposite direction for 1 minute. This procedure was repeated again and then the entire volume of elution solution and microbial contents were collected and the volume measured. A volume of eluate was removed (approximately 10 mL) for analysis of coliphage quantities as described above. The remaining volume was filtered through a 0.22 μm membrane filter. Two hundred microliters was used in DNA extraction using the QIAamp DNA Blood Mini Kit following the manufacturer's instructions with the substitution of Buffer AVL (Qiagen) with modifications described above. DNA was eluted from mini columns with 3–50 μl elution steps. Quantities of adenovirus was determined by qPCR as described above.

To assess the recovery efficiency of the targeted microbes through the ultrafilter processing steps, 9 L of MBR effluent was seeded with 1 L of primary effluent collected from a local WWTP plant the morning of each evaluation. The seeded sample was mixed for 10 minutes on a stir plate, and then 20 mL was removed. One 10 mL aliquot was stored at 4 °C for coliphage analysis and another 10 mL was stored at −20 °C for DNA extraction. The sample was filtered through an ultrafilter using the peristaltic pump set at 300 RPM. The ultrafilter was eluted and microbes assayed as described above. Recovery efficiency was determined by dividing the number of targeted microbes measured after processing by the number measured before processing and multiplying by 100.

During the spiking study (described in section 2.7), effluent grab samples were taken and analyzed for coliphage as described.

2.5 Natural operation of MBR

The MBR system was monitored for a total of 114 days by the online sensors. During this period, 105 days were considered "natural operations" and 9 days were considered "experimental operations." For this study, operation was considered "natural" except when the MLSS bypass and spiking trials were being performed (discussed in sections 2.6 and 2.7). In addition to collecting sensor data and monitoring traditional wastewater characteristics, twice-weekly composite effluent samples were collected to assess concentrations of adenovirus (n = 13 samples) and somatic and male-specific coliphage (n = 14). Additionally, grab samples of the influent feed water and MLSS were taken to assess the concentrations of adenovirus (n = 5) and somatic and male-specific coliphage (n = 7) to determine the background viral presence.

2.6 Membrane bypass experiments

Bypass experiments were performed to test how online sensors and CANARY would respond to known contamination events. Alarms that occurred during the bypass

experiments were considered to be associated with those experiments (*i.e.*, true positives). These bypass experiments were intended to mimic a membrane integrity failure which could result in poor effluent water quality.[30] During these experiments (n = 6), MLSS was pumped directly into the effluent lines preceding all of the sensors at a rate of 1 mL min^{-1}, or approximately 2% of the effluent flow, for 7 hours. Pumping of the MLSS directly into the effluent line was preferable to avoid permanently damaging the membrane. During the final hour of the bypass experiments, an ultrafilter was installed within the effluent line to collect a 3 L composite sample. The ultrafilter was then processed for adenovirus and somatic and male-specific coliphage as described above.

2.7 MS2 spiking experiment

A spiking experiment was conducted to simulate high viral loading that might occur in a wastewater reuse system. Bacteriophage MS2 was chosen as the viral surrogate because of its size and structural similarities to viral pathogens.[7] The spiked concentration was expected to overload the MBR, resulting in breakthrough of viral organisms into the effluent. To culture the MS2, *E. coli* (HS(pFamp)R, ATCC700891) was grown to mid-log phase in tryptic soy broth amended with 0.015 mg mL^{-1} each streptomycin and ampicillin at 37 °C. MS2 stock (ATCC 15597-B1) was added and incubated for approximately 16 hours at 37 °C. MS2 coliphage was harvested by centrifugation of the culture at 3300g for 15 minutes followed by filtration through 0.22 μm filter. MS2 coliphage were enumerated using the double agar layer method described above.

MS2 coliphage were injected into the reactor and supplemented in the influent to achieve a concentration of approximately 10^5 PFU mL^{-1}, at a rate of 1 mL min^{-1} to compensate for the MS2 loss due to daily reactor sludge wasting, lasting for 36 hours. Grab samples of the influent and MLSS were collected 30 minutes before starting the MS2 spike and again at 0.5, 4, 6, 9.5, 24, and 30 hours after the MS2 spiking commenced. Additionally, effluent grab samples were collected at 4, 6, 9.5, 24, and 30 hours after the MS2 spiking commenced. Finally, an ultrafilter was placed in the effluent line at 24 hours and 30 hours after MS2 spike was introduced for 6 hours each. Removal of MS2 was calculated as $\log_{10}(N_x/N_0)$, where N_x is MS2 concentration (PFU mL^{-1}) at a time = X and N_0 is the MS2 concentration (PFU mL^{-1}) at time = −0.5.

3. Results and discussion

3.1 Reactor operation

After the initial seeding of activated sludge from a local wastewater treatment plant, the reactor was allowed to stabilize. During the stabilization and experimental periods, traditional wastewater characteristics were monitored to ensure the MBR was functioning properly. At the end of the stabilization period and throughout the experimental period, the MBR removed an average of 85% COD, >99% NH_3–N, and 96% TKN. These data suggest that the MBR was operating as expected.

3.2 Monitoring during natural conditions of the MBR

CANARY analyzed sensor data from the MBR effluent for a total of 114 days, of which 105 days were during natural operation while the other 9 days were simulated failure conditions of the MBR. CANARY analyzes a continuous stream of data, which results in alarm behavior based on the entire timeframe of data analyzed. Table 1 categorizes all CANARY alarms by the type of event that was associated with each alarm. All alarms that occurred outside of simulated failure condition periods were categorized as natural alarms, and further subdivided by what type of event was occurring during the alarm timeframe. Of the 239 total alarms, 219 occurred during the period of natural operating conditions, and 196/219 (89%) of those alarms could be attributed to an event in which the sensor changes were the result of an operator-induced disruption. These disruptions were grouped into 4 categories: MBR Operation Events (*i.e.*, TMP exceedances, membrane cleaning, *etc.*), Sensor Operation Events (*i.e.*, sensor calibration or maintenance, *etc.*), Data Collection Events (*i.e.*, addition of ultrafilter inline to obtain a composite sample for microbial analysis) and Feed Composition Change (*i.e.*, changes in mined wastewater due to weather events, University events, presence/absence of student body, *etc.*). The 23/219 (11%) of alarms that did not coincide with a known system disruption were considered to have an unknown origin. Of the 23 unknown alarms, 10 unknown alarms were the result of short-lived (less than 30 min) changes to a sensor signal. These short-lived alarms were likely influenced by the scale and unique sensor setup of this system and are not likely to be indicative of poor water quality events. The remaining 13 alarms corresponded to prolonged sensor disruptions (*i.e.*, greater than 30 min) in 2 or more of the CDOM, UV_{254}, DO, turbidity or TOC sensors, which may be related to reduced effluent water quality. These alarms are discussed in greater detail below.

Examples of the sensor and CANARY outputs for two days of natural operating conditions are shown in Fig. S1 and S2.† Fig. S1† shows a day with no known events where the sensor deviations were small within the selected 15 minute BED window (BED window = 30) and did not yield a CANARY alarm. The sensor change and subsequent CANARY alarm observed in Fig. S2† was attributed to a feed composition change. The 13 alarms associated with feed composition changes during this study were likely influenced by the benchtop scale of this MBR system, and the unique, localized sewer mining techniques utilized in this study, which resulted in changes in feed quality related to weather events, student presence, and University events. Therefore, these issues may not be present in a system that is larger or has a more stable feed composition. Since no samples were taken for assessment of microbial quality when feed composition changes were observed, no conclusions can be made related to any possible increase in risk of poor effluent quality during these periods. Sensor responses were similar to those observed during the spike and bypass experiments described below, so future investigations into larger scale application is warranted.

One of the goals of this study was to determine if CANARY alarms had any association with viral presence in treated effluent. Therefore, throughout natural operation, influent ($n = 7$) and effluent ($n = 14$) samples were collected and assayed for concentrations of adenovirus and somatic and male-specific coliphage. These samples were taken to establish baseline concentrations and removals for this system, and to aid in comparison during the bypass and

spike studies. Influent samples were assayed as grab samples without concentration, but effluent samples were concentrated to increase sensitivity of detection. Evaluation of this concentration procedure with raw sewage from a local WWTP spiked into MBR effluent (n = 5) revealed 66 (±20), 93 (±17), and 98 (±49) % recovery efficiency of somatic and male-specific coliphage, and adenovirus, respectively. Influent mean concentrations of somatic and male-specific coliphage were 79 and 95 PFU mL^{-1}, respectively (Table 2). In the effluent composite samples, 0–0.01 PFU mL^{-1} of somatic coliphage were detected (14% of samples were positive), but male-specific coliphage was not detected in any sample. These data suggest that the MBR removed greater than 2-log_{10} of coliphage, but low influent levels prohibited quantification of removal. Adenovirus was present in low levels in some influent (40%) and effluent (8%) samples, but a lack of data prohibited removal calculations.

3.3 Bypass experiments

Six MLSS bypass trials were performed to simulate a membrane integrity failure. All six trials were detected by CANARY. Fig. 2 contains the sensor output associated with the fifth bypass experiment, and is representative of the other bypass experiments. Each of the bypass experiments began at 10:00 AM. The sensor signals began to respond as effluent with mixed liquor reached the sensors after approximately 10:45 AM (consistent with the travel time expected for the sensor configuration used). The deviation in sensor signals increased after 11 AM and CANARY reported alarms, shown at 11:22 AM for the fifth trial (Fig. 2). The total delay between the beginning of the bypass and the CANARY alarm is an artifact of the configuration of the system—where the MBR effluent has to pass through all the sensors in series. Previous uses of CANARY reported response times of 30 minutes or less when using 5 minute or shorter data,[26] which is consistent with the current study when initial sensor response timing is considered.

For all six trials, the turbidity and TOC sensors showed the most significant deviations, but the other sensors including UV_{254} and CDOM also contributed to the bypass-related alarms. While the deviation in the UV_{254} signal is clear in Fig. 2, the change in the CDOM signal is subtle and may have gone unnoticed with visual inspection alone. The DO sensor did contribute to CANARY alarms during the bypass study, but not as prolonged or as consistently as the other signals. Fig. 2 also shows two alarms beginning at approximately 3 AM and 6 AM as well as an alarm beginning approximately at 4:45 PM. The first two alarms are in response to the MBR backflush cycle discussed in section 3.1. CANARY could have been configured to eliminate alarms related to the cleaning cycle, but not without possibly reducing true positive rates. It was also not clear whether those membrane scouring events could negatively impact effluent water quality.[31] The alarm at 4:45 PM was due to the installation of an ultrafilter for the collection of microbial data (listed as a bypass event in Table 1). The sensor behavior exhibited in Fig. 2 at 4:45 pm is typical of the ultrafilter installations and was attributed to washout of the preservative solution in the ultrafilters. An ultrafilter was installed for all 6 of the bypass trials, all of which resulted in a CANARY alarm.

During these trials, adenovirus and somatic and male-specific coliphage were measured in the influent, MLSS, and effluent (Table 3). One goal of these simulated membrane failure

experiments was to determine if any changes in water quality measured by the online sensors and CANARY corresponded to virus (surrogate or pathogen) presence. Adenovirus and somatic and male-specific coliphage were present in 0 (0%), 5 (83%), and 0 (0%) of effluent samples, respectively, collected during the alarm periods. Trial 3 was the only trial in which no virus was present in the effluent, which was likely due to lower influent levels observed during this experiment. By comparison, during natural MBR operation, adenovirus and somatic and male-specific coliphage were present in 1 (8%), 2 (14%), and 0 (0%) of effluent samples, respectively. On average, when detected, somatic coliphage was approximately 2 orders of magnitude higher in the effluent samples during the bypass studies than during natural operations. These results are similar to those found in other studies investigating membrane breaches in MBRs. For example, Hirani *et al.*[32] caused a membrane breach by puncturing a flat-sheet membrane (3 cm long × 2–4 mm wide) in pilot-scale MBR, and found higher levels of both total coliform bacteria and coliphage in the effluent after breaching than before.

The results of these bypass experiments suggest that the online water quality sensors and CANARY software were able to detect the change in water quality signals caused by the addition of 2% unfiltered MLSS. In addition, there was increased viral presence found in the effluent of the bypass experiments compared to that found during natural operation. Although no sensor used in this study could directly detect viruses in real-time, CANARY was able to identify the changes in water quality that were associated with poor treatment which corresponded to viral presence. These results suggest that CANARY could be implemented as part of an early warning system to assist operators with identifying treatment malfunctions in water reuse systems.

3.4 Spiking experiment

Bacteriophage MS2 was used as a viral surrogate in these experiments to determine the sensor and CANARY response to high levels of viruses that may be present in water reuse systems during a viral outbreak.[33] Given the expected 4-$\log_{10}$ reduction of male-specific phage through an MBR,[31,34] the goal was to spike the reactor with MS2 levels sufficient for continued detection of culturable phage in the effluent.

During the MS2 spiking trial, the reactor was spiked to a concentration of 10^5 PFU mL^{-1} of MS2, and was maintained at this concentration by supplementing MS2 in the influent for 36 hours (Fig. 3). Influent and effluent grab samples were taken at defined time points (Table 4) to collect microbial data. During the first 10 hours of spiking, the MBR exhibited a 2.5–2.9-$\log_{10}$ reduction of MS2 which is less than the expected 4-$\log_{10}$ removal, but similar to $\log_{10}$ removals reported previously for pristine or chemically cleaned membranes.[31,34]

CANARY generated an alarm in response to the MS2 spike within the expected HRT of the MBR, consistent with the occurrence of the alarms during the bypass experiments. Fig. 3 contains the sensor outputs, CANARY outputs, and the results of the microbial analysis associated with this spiking trial. All sensors included in the CANARY analysis except conductivity contributed to the CANARY alarm at 8 AM. The alarm at approximately 10 PM can be attributed to the system stabilizing near baseline values. This type of alarm behavior was expected, and can be modified by using a different event timeout parameter

value in CANARY. During the 8 AM alarm period, the TOC sensor increased from 5 mg L^{-1} up to 15 mg L^{-1} and the CDOM sensor dropped to approximately 3000 fluorescent units before increasing to approximately 3800 fluorescent units. The majority of the sensors likely responded to the chemical composition of the culture medium as opposed to direct detection of the MS2. Detection of the culture medium was not surprising given that it has been observed in other studies investigating real-time detection of *E. coli* in water.[15,35]

The MS2 concentration in the effluent remained fairly stable from 12–5 PM (~10–40 PFU mL^{-1}), but most sensor signals appear to be returning to baseline during this time. The CANARY alarm was reported from approximately 8–11 AM, but was turned off after 500 timesteps (250 minutes) as set in event threshold parameter. The CANARY response captures the initial signal change following the high viral loading was followed by a slow return to baseline as the MBR adjusts to feed change. Sensor data suggests that the disruption to the system was in the final stages of exiting the system at 5 PM; however, the concentration of the MS2 virus was measured at 19 PFU mL^{-1} which may have still posed a potential threat to public health at that time.

4. Conclusions

During the 114 day study period, CANARY generated 239 total alarms, 20 of which occurred during simulated failures. CANARY accurately generated an alarm during all bypass and spike challenge experiments. Of the remaining 219 alarms, 196 (89%) could be attributed to operator-induced events such as membrane cleaning or sensor maintenance, or system events such as drastic changes to feed composition. The remaining 23 (11%) alarms that occurred during natural operations were considered unknown. Of the 23 alarms with an unknown source, there were signal changes in 13 that were similar to other alarms with a known cause suggesting a possible decrease in effluent water quality. Future research could encompass automatic sampling triggered by CANARY alarms to confirm decreased effluent water quality.

CANARY successfully identified the 2% bypass of the MLSS directly into the effluent line in all 6 trials as well as the MS2 spiking trials. However, in the spiking trial, the sensors were likely reacting to the culture medium as opposed to direct detection of the phage. Future research could evaluate the sensors and CANARY's response by separating the phage from its host's growth medium. The timely detection of simulated treatment failures by CANARY supports its use as part of an early warning detection system for water reuse systems. However, a majority of the alarms that occurred in our system during natural operating conditions that were attributed to operator or system events are likely unique to this system setup. An application of a similar detection approach in Australia found fewer false alarms in a full-scale evaluation at a wastewater treatment plant;[17] however, the approach did not address microbial or viral detection. Therefore, future research could apply CANARY in larger systems and other types of water reuse systems to evaluate its applicability in these systems. Further testing is also warranted to determine if real-time sensors other than those used herein are capable of providing other meaningful parameters in the context of monitoring treatment operation in water reuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The research has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.

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Water impact

Timely monitoring practices in decentralized water reuse systems is needed to ensure treated water is safe for consumers at the point-of-use. On-line physical and chemical sensors coupled with CANARY event detection software can be a useful tool for providing early indications that the treated water quality has diminished, thereby allowing operator intervention prior to end-user exposure.

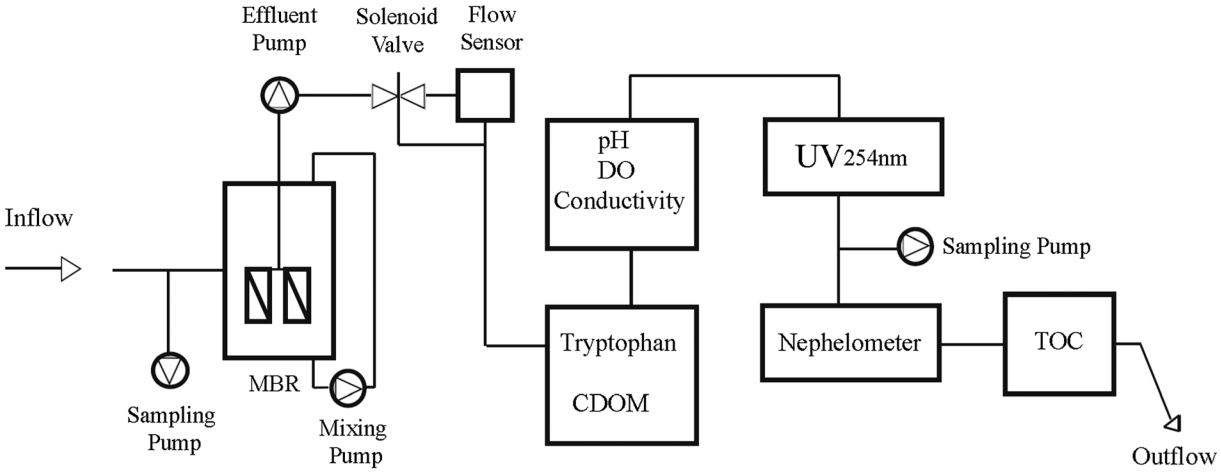

Fig. 1.
Schematic of the MBR and online monitoring setup.

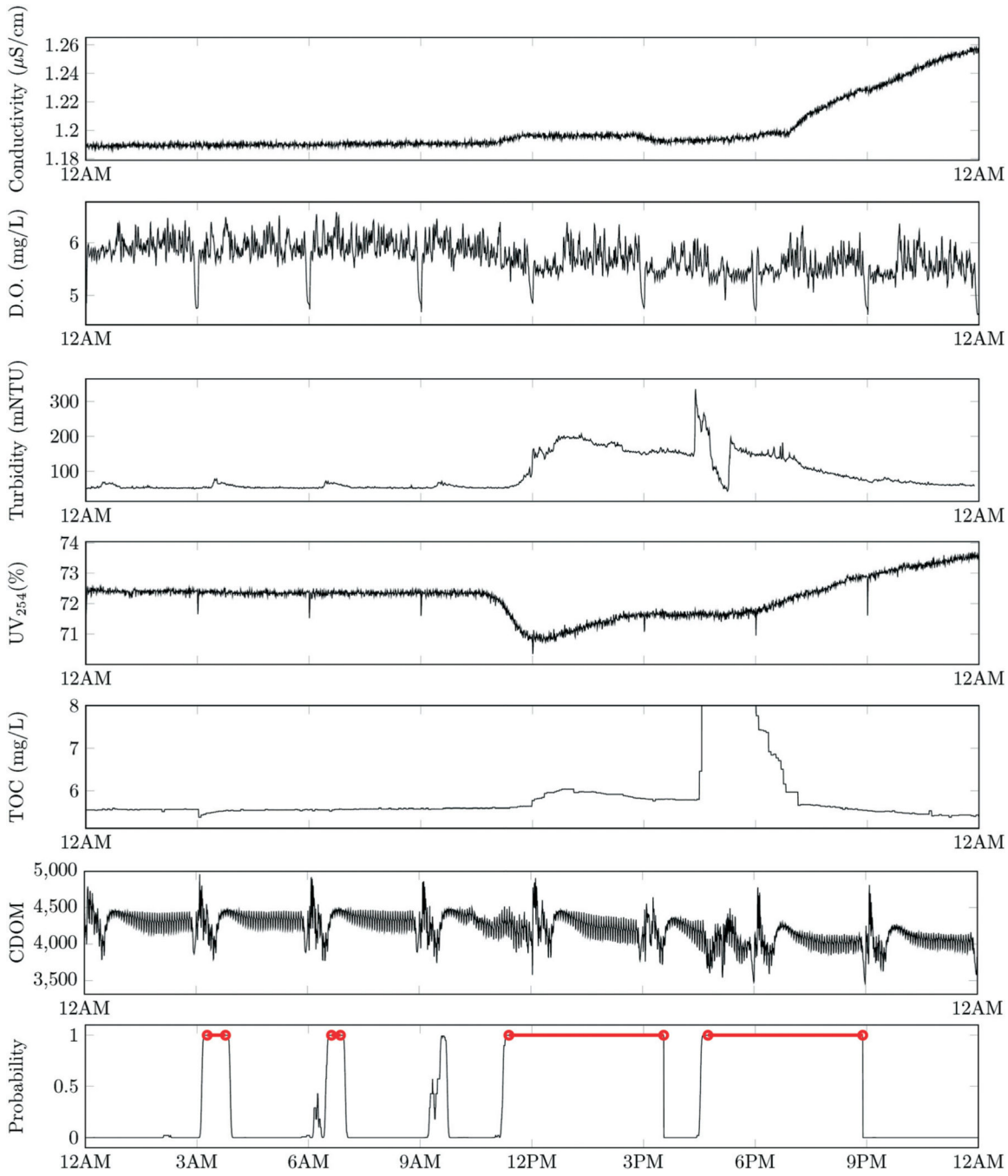

Fig. 2.

Sensor response and CANARY assessment to the fifth experimental trial of the bypass of the MLSS (commenced at 10 AM) and installation of a hollow fiber ultrafilter (installed at 4 PM). Alarms at 3 AM and 6 AM are due to the MBR backflush cycle.

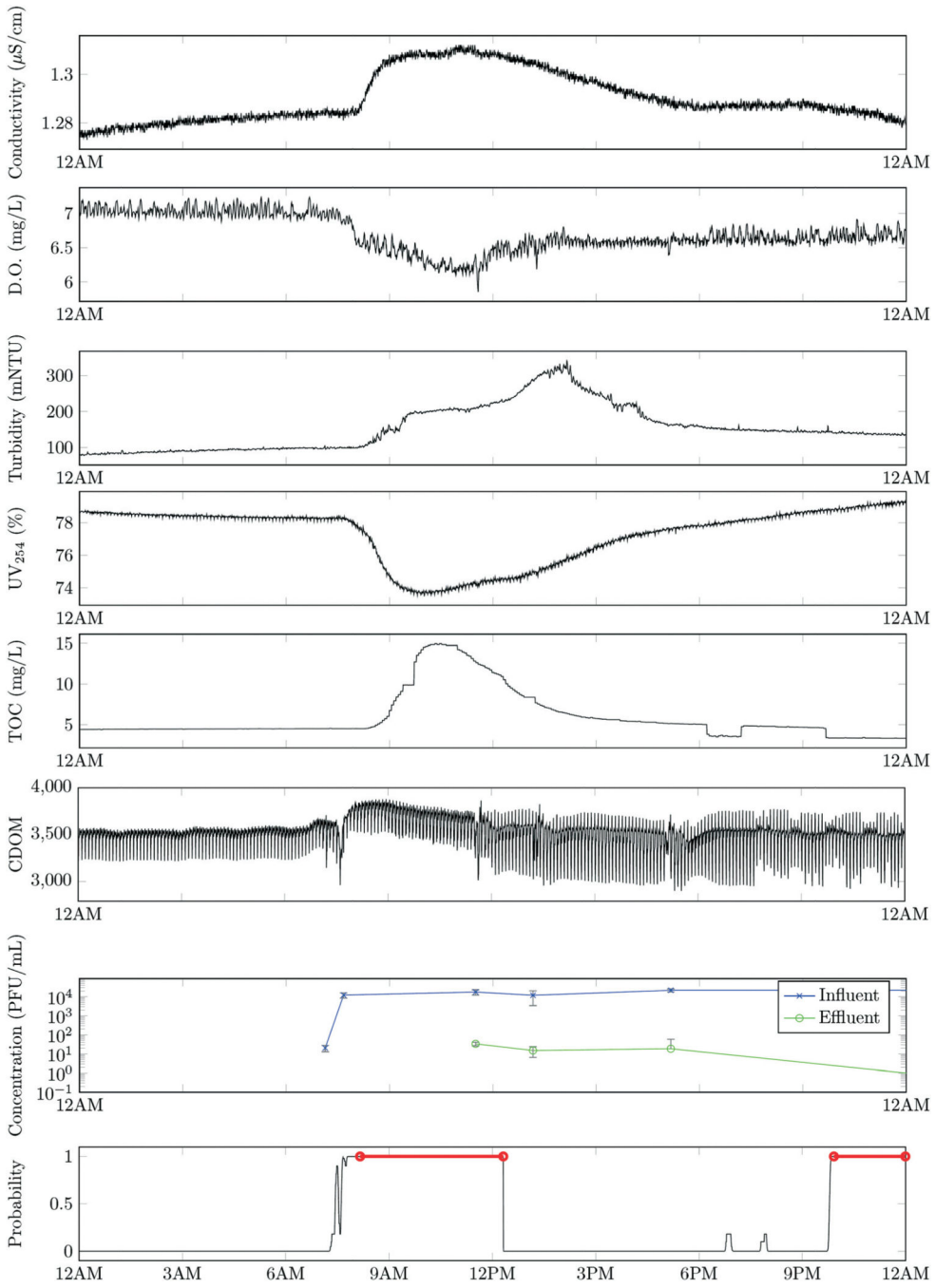

Fig. 3.

Sensor response, CANARY assessment and MS2 measurements during the MS2 spiking experiment. Alarm at 10 PM is due to the sensors returning to baseline values.

Table 1

CANARY alarms

Operational time period	Event classifications	CANARY alarms
Natural	MBR operation events	114[a]
	Sensor operation events	46[b]
	Data collection events	23[c]
	Feed composition change	13
	Unknown events	23
Experimental	Bypass events	12
	Spike events	8
Total		239

[a]13 alarms were caused by multiple events.

[b]10 alarms were caused by multiple events.

[c]7 alarms were caused by multiple events.

Table 2

Targeted microbe measurements during natural MBR operation

	Adenovirus (gc mL^{-1})		Somatic coliphage (PFU mL^{-1})		Male-specific coliphage (PFU mL^{-1})	
Date	**Influent**	**Effluent**	**Influent**	**Effluent**	**Influent**	**Effluent**
4/1/2015	—[a]	0	23	0	72	0
4/3/15	—	0	—	0	—	0
4/8/15	—	0	—	0	—	0
4/10/15	—	0	29	0	60	0
4/15/15	—	0	—	0	—	0
4/22/15	—	0	—	0	—	0
5/1/15	0	0	17	0	208	0
5/6/15	—	—	—	0	—	0
5/8/15	10	0.189	84	0	212	0
5/26/15	—	0	—	0	—	0
5/28/15	0	0	6	0	16	0
6/2/15	—	0	—	0	—	0
6/4/15	14	0	337	0.0046	76	0
7/8/15	0	0	60	0.0112	18	0

[a] A dash (—) indicates the assay was not performed.

Table 3

Targeted microbe measurements during bypass studies

	Adenovirus (gc mL^{-1})			Somatic coliphage (PFU mL^{-1})			Male-specific coliphage (PFU mL^{-1})		
Trial	**Influent**	**MLSS**	**Effluent**	**Influent**	**MLSS**	**Effluent**	**Influent**	**MLSS**	**Effluent**
1	—	—	0	—[a]	—	0.313	—	—	0
2	0	0	0	139.33	65.16	0.464	45.5	4.67	0
3	3.278	0	0	17.4	29.67	0	23.67	0.67	0
4	3.2	3.28	0	130	146.67	1.2	—	—	—
5	0	0	0	23	34.83	0.1	0	0	0
6	7.78	0	0	81	32.2	0.096	46.5	5.5	0

[a]A dash (—) indicates the assay was not performed.

Table 4

MS2 coliphage measurements during spiking study

	MS2 coliphage (PFU mL^{-1})	
Time[a] (h)	**Influent**	**Effluent**
−0.5	21	—[b]
0.5	8117	—
4	11 800	34
6	11 900	15
10	14 567	19
24	14 600	0.05[c]
30	24 450	0.01[c]

[a] Time relative to start of MS2 spike.

[b] A dash (—) indicates the assay was not performed.

[c] MS2 was not detected in the grab sample, but in the ultrafilter concentrated sample.