THE METROPOLITAN WATER DISTRICT
OF SOUTHERN CALIFORNIA

THE Water Research FOUNDATION

PROJECT NO.
5041

Demonstrating Virus Log Removal Credit for Wastewater Treatment and Reverse Osmosis for Potable Reuse at OCWD

CITY OF FULLERTON CALIFORNIA

ORANGE COUNTY WATER DISTRICT
SINCE 1933

Demonstrating Virus Log Removal Credit for Wastewater Treatment and Reverse Osmosis for Potable Reuse at OCWD

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Co-sponsored by:

City of Fullerton

Metropolitan Water District of Southern California

Orange County Water District

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Metropolitan Water District Future Supply Actions Funding Program

Metropolitan's FSA Program supports long-term resource diversification and prepares the region for future water supply uncertainties. Funded projects are intended to evaluate new water treatment technologies, develop data to inform regulations, study options for infrastructure innovation, and identify future resource potential.

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Acronyms and Abbreviations

AS	Activated sludge
AS1	Activated sludge process train 1
AS2	Activated sludge process train 2
ATP	Adenosine triphosphate
AWPF	Advanced water purification facility
BCS	Biological Consulting Services
BOD-t	Total biological oxygen demand
BGM	Buffalo Green Monkey
BWW	Backwash waste
CPE	Cytopathic effects
ddPCR	Droplet digital polymerase chain reaction
DDW	Division of Drinking Water
DPR	Direct potable reuse
EC	Electrical conductivity
EEM	Excitation-emission matrix
EPA	U.S. Environmental Protection Agency
fDOM	Fluorescing dissolved organic matter
GC	Gene copies
GC/L	Gene copies per liter
GWRS	Groundwater Replenishment System
GWRSFE	Groundwater Replenishment System Final Expansion
HPOAS	High purity oxygen activated sludge
IAP	Independent Advisory Panel
IC	Ion chromatography
IPR	Indirect potable reuse
IQR	Interquartile range
LRV	Log removal value
MC	Monte Carlo
MCRT	Mean cell residence time
MFE	Microfiltration effluent
MFF	Microfiltration feed
MFGM	Membrane Filtration Guidance Manual
MGD	Million gallons per day
MLTSS	Mixed liquor total suspended solids
MPN	Most probable number
MPN/L	Most probable number per liter
MS	Male-specific (F+) coliphage

MSU	Michigan State University
MSR	Matrix spike recovery
NCWRP	North City Water Reclamation Plant
NDN	Nitrification-partial denitrification
NPDES	National Pollutant Discharge Elimination System
NTA	Nanoparticle tracking analyzer
NWRI	National Water Research Institute
OC San	Orange County Sanitation District
OCWD	Orange County Water District
ORV	Operating range value
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PFU/L	Plaque forming units per liter
P1	OC San Plant No. 1
P2	OC San Plant No. 2
RO	Reverse osmosis
RNA	Ribonucleic acid
SARI	Santa Ana Regional Interceptor
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SEFE	Secondary effluent flow equalization
SOM	Somatic coliphage
SRT	Solids retention time
SWRCB	California State Water Resources Control Board
TF	Trickling filter
TF/SC	Trickling filter/solids contactor
TOC	Total organic carbon
TSS	Total suspended solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

Executive Summary

Indirect potable reuse (IPR) and direct potable reuse (DPR) projects must meet pathogen removal requirements to ensure protection of public health. The Groundwater Replenishment System (GWRS) is a joint IPR project of the Orange County Water District (OCWD) and the Orange County Sanitation District (OC San). The GWRS Advanced Water Purification Facility (AWPF) treats secondary treated wastewater from OC San facilities to produce 100 million gallons per day (MGD) of highly purified water from wastewater that would otherwise be discharged to the ocean. The AWPF treatment train is comprised of microfiltration (MF), reverse osmosis (RO), an ultraviolet advanced oxidation process (UV/AOP) that includes disinfection by hydrogen peroxide addition, and decarbonation followed by lime stabilization. Following advanced treatment, this highly purified water is injected into a nearby seawater intrusion barrier and pumped along a 14-mile pipeline for delivery to OCWD's injection wells and spreading basins for groundwater replenishment. The AWPF and related groundwater replenishment infrastructure is operated by OCWD to recharge and augment the region's drinking water supply stored in the Orange County Groundwater Basin.

In 2014, the California State Water Resources Control Board (SWRCB) Division of Drinking Water (DDW) finalized regulations for IPR via groundwater recharge. These regulations established the requirement of high log reduction values (LRVs) of 12/10/10 for viruses, *Giardia,* and *Cryptosporidium*, respectively. Accordingly, advanced water treatment facilities such as OCWD GWRS must demonstrate that the required removals for viruses (12 logs or 99.9999999999%) and protozoa (10 logs) are met between the source raw wastewater and the finished groundwater produced from downgradient drinking water wells. Log removal credits can be assigned to different unit treatment processes that occur between these two points.

OCWD currently receives one log virus credit for every month underground between the spreading locations where purified water is infiltrated and the drinking water well production sites. Therefore, currently the GWRS requires four months of underground retention time to meet the 12-log enteric virus removal requirement of the California groundwater augmentation regulations, since only ~8.2 logs of virus removal credit are currently obtained for the advanced water treatment. Demonstrating additional LRVs at treatment stages preceding UV/AOP would reduce the required underground travel time and, therefore, increase the number of viable future injection sites, and/or allow for additional credits as a safety factor (since currently typically ~12.2 logs are credited compared to the 12 required for viruses).

This report presents the approach and outcomes of a microbial monitoring study of the wastewater treatment process that precedes GWRS and, separately, an integrity surrogate monitoring study to assess RO performance, which were completed to propose additional regulatory virus log removal credits for the GWRS to supplement existing credits to demonstrate greater log removal.

The objectives of this study were to:

- Measure the concentration and removal of enteric viruses and microbial indicators for four OC San treatment processes that produce secondary treated effluent that serves (or will serve) as GWRS influent,
- Evaluate and select the most suitable statistical approach to determine and propose a single log removal credit value for the wastewater treatment process to apply toward the 12 log virus removal requirement, as well as propose plant operational performance metrics for parameters that are monitored daily on which virus credit would be contingent, and
- Evaluate promising candidate surrogates for monitoring RO integrity for potable reuse that are present in RO feed water to demonstrate and propose higher log removal credit for viruses over traditional process indicators such as total organic carbon (TOC) and electrical conductivity (EC).

The approach and findings of the wastewater treatment process microbial monitoring study are discussed in Chapters 2 through 7. A virus monitoring study that consisted of 24 sampling events was conducted for raw wastewater (OC San influent) and secondary effluents from OC San's two wastewater treatment plants. Data collected from these sites were analyzed using two alternative statistical approaches including a Monte Carlo simulation and a covariance approach to calculate virus log removal values. Sampling design for the microbial monitoring study used established strategies based on experience from other studies in addition to standardized methodologies to calculate virus log removal for the wastewater treatment process. Based on the covariance approach, a conservative 5th percentile LRV of 0.73 (82% removal) was proposed to DDW as the virus log removal credit value that represented the lowest 5^{th} percentile LRV of the four wastewater treatment processes monitored. DDW recognizes the 5^{th} percentile LRV as the preferred statistically conservative pathogen removal value, as opposed to the mean or median (50^{th} percentile).

Details on the approach and findings for integrity surrogates for monitoring RO performance are discussed in Chapters 8 and 9. RO has traditionally been, and still is, under-credited for pathogen removal due to the lack of an online, near-real-time, or daily grab sample-based monitoring strategy to continuously demonstrate membrane and system integrity at levels close to actual expected pathogen removals. To address this issue, an evaluation of integrity surrogates for monitoring RO performance was performed at the OCWD AWPF on one of the full-scale 5-MGD RO units. Promising candidate surrogates were identified and a total of five surrogates was monitored including strontium, sulfate, free adenosine triphosphate (ATP), nanoparticles, and fluorescence Peak C, to assess their feasibility to replace the traditional surrogates TOC and EC.

Key observations from this study include:

- The covariance statistical approach can be used to calculate virus log removal from rank paired influent and effluent microbial concentration datasets.
- The OC San Plant No. 1 trickling filter process had the lowest performance for virus removal with median and 5^{th} percentile LRVs of 1.0 (90%) and 0.73 (82%) for cultivable enteric viruses, respectively.

- For the RO process, strontium, sulfate, and free ATP showed the highest removal with average LRVs of 3.29, 2.97 and 3.03, respectively.

In conclusion, OCWD anticipates being awarded virus credit toward the wastewater treatment process (currently granted no credit), as well as enhanced credit for the RO process as a successful outcome of this study. For wastewater treatment, the calculation approach is being reviewed by DDW at the time of this report. For the RO process for the GWRS, OCWD has proposed to DDW to use strontium, sulfate, and/or free ATP in a tiered approach as the primary surrogates for virus LRV credit while maintaining TOC and EC monitoring as back-ups and for other performance monitoring purposes. The implementation of the particular RO crediting approach and monitoring program is still being finalized and reviewed by DDW. Results from this study were incorporated into the Title 22 Engineering Report related to permitting the OCWD GWRS Final Expansion to (1) propose a conservative 5th percentile LRV for the wastewater treatment process toward virus removal credit requirements that is contingent on OC San processes meeting daily performance metrics for key plant operational parameters; and (2) propose a tiered combination of strontium, sulfate, and/or ATP as the primary integrity surrogates for the RO process whereby virus LRV credit is based on the daily average LRV for the surrogate compound.

ES.1 Related WRF Research

- Advancing Safety and Reliability to Protect Public Health: Identifying Quantitative Reductions of Viral Pathogens and Surrogates for Water Reuse Applications (5126)
- Demonstration of Pathogen Removal Credits in Wastewater Reuse: 21st Century Guidance Materials for Study Plans and Reporting (5047)
- Indicator Viruses for Advanced Physical Treatment Process Performance Confirmation (4955)
- Operational, Monitoring, and Response Data from Unit Processes in Full-Scale Water Treatment, IPR, and DPR (4767)

CHAPTER 1

Project Background

The objective of this study was to obtain increased regulatory "credits" toward virus log removal value (LRV) requirements for potable reuse via two key strategies: (1) appropriately crediting the engineered wastewater treatment process that precedes advanced treatment and (2) demonstrating reverse osmosis (RO) process integrity as part of advanced treatment via enhanced monitoring. The study sites were the Orange County Sanitation District (OC San) which operates two wastewater treatment facilities and the Orange County Water District (OCWD) which operates an Advanced Water Purification Facility (AWPF) as part of the Groundwater Replenishment System (GWRS) for potable reuse. GWRS is a joint project of OC San and OCWD. Through this project, purified, recycled water is continuously recharged to groundwater as a major component of the local drinking water supply in north and central Orange County, California. The purified water is also used by OCWD to maintain a seawater intrusion barrier (via a series of injection wells) to protect the groundwater quality.

This report is thus presented in two parts. Chapters 2 through 7 present the approach and findings of the wastewater treatment evaluation. An approximately two-year enteric virus monitoring study was conducted for raw wastewater (plant influent) and secondary treated effluent at OC San's two wastewater treatment plants that serve secondary effluent as source water to the OCWD GWRS potable reuse facility. Two statistical methods were applied and compared to calculate log removal of virus for each monitored treatment process and to propose a conservative value for the log credit. Chapters 8 and 9 present the approach and findings of the RO process evaluation. Promising surrogates for demonstrating RO integrity toward pathogen LRV credit were evaluated for the AWPF RO process, including for some novel surrogates not previously studied. At the time of this report, OCWD has submitted proposals to the California State Water Resources Control Board (SWRCB) Division of Drinking Water (DDW) to request virus log removal credit for the wastewater treatment and pathogen log removal credit (virus, *Giardia*, and *Cryptosporidium*) for the RO process based on information developed during this study.

1.1 Regulatory Requirements for Pathogen Removal Credit

In 2014, the California SWRCB DDW finalized regulations for indirect potable reuse (IPR) via groundwater recharge. A large degree of conservatism compelled the establishment of high required log reduction values (LRVs) of 12/10/10 for virus, *Giardia* and *Cryptosporidium*, respectively. Therefore, advanced water treatment facilities such as OCWD GWRS must demonstrate that the required removal for virus (12 logs or 99.9999999999%) is met between the source wastewater and uptake by downgradient potable drinking water wells. Facilities must validate through a process-specific study or challenge test that each treatment process used for pathogen LRV credit can reliably and consistently achieve the targeted log reduction. For the advanced purification processes, these validated process-specific LRV credits must then be verified through on-going process control monitoring using either a pathogenic microorganism of concern or surrogate parameter. The OCWD GWRS pathogen monitoring

program is based on surrogate measurements through each unit process within its advanced water purification facility.

1.2 OCWD GWRS Facilities Description

The GWRS is a potable water reuse project featuring advanced purification of secondary wastewater to achieve a potable quality for groundwater recharge of the Orange County Groundwater Basin in Southern California. This recycled water supply is critical for the region, representing more than 30% of the groundwater recharge carried out by OCWD, and supplements other limited local supplies and imported water supplies for regional drinking water.

The major components of the GWRS include the AWPF, pump stations, and the pipelines that convey the purified recycled water to OCWD's recharge basins and injection wells. The AWPF is located adjacent to OC San Reclamation Plant No. 1 (P1) in Fountain Valley (Figure 1-1). The finished water produced by GWRS AWPF supplements existing water supplies by providing a reliable, high-quality source of water to recharge the Orange County Groundwater Basin and to protect the groundwater basin from degradation due to seawater intrusion. GWRS has been in operation since January 2008. The original AWPF had a treatment capacity up to 70 million gallons per day (MGD), which was subsequently increased in 2015 to 100 MGD. The GWRS Final Expansion will increase the AWPF capacity to 130 MGD and is scheduled to be completed in 2023.

Figure 1-1. Location of OCWD's GWRS Facility and OC San's Wastewater Treatment Plants No. 1 and No. 2.

1.3 Need for Enhanced Credits at OCWD GWRS

Without the enhanced credits that may be awarded as a result of this project, OCWD GWRS currently achieves the pathogen removal credits shown in Table 1-1. GWRS requires four months of underground retention time to meet the 12-log enteric virus removal requirement of the California groundwater augmentation regulations, since only ~8.2 logs of virus removal credit are currently obtained for the advanced water treatment (SWRCB DDW 2017, OCWD 2015). Thus, the underground retention time for the purified water in the storage aquifer is an important component. OCWD currently receives one log virus credit for every month underground between the spreading locations where purified water is infiltrated and the drinking water well production sites. Based on the sum of AWPF credits plus underground retention time, OCWD achieves the required 12-logs for virus with only a small excess above the minimum 12 (e.g., ~12.2 logs) depending upon the particular daily monitoring result for total organic carbon (TOC) rejection across RO which is generally just above 2 logs. This leaves little room for error (e.g., a TOC sensor issue), which led OCWD to install dual TOC online analyzers on both the RO feed and permeate locations for redundancy.

Further, demonstrating additional LRVs would reduce the underground travel time required to reach 12 logs. This would have significant practical implications and benefits for OCWD (and other IPR utilities), by increasing the number of viable future injection and recharge sites for groundwater recharge.

Because an excess of *Giardia* and *Cryptosporidium* are currently achieved (Table 1-1) for GWRS above the 10-log requirement, the present study did not include these microbial targets in the wastewater treatment study, focusing that effort solely on measuring enteric virus removal with some complimentary microbial indicator sampling. Separately, while virus log credit was also the driver for the RO process study, any enhanced credit demonstrated for RO for virus is expected to extend to *Giardia* and *Cryptosporidium* credits as well, further increasing the total credits for these pathogens. This is because *Giardia* and *Cryptosporidium* are larger than virus particle sizes, and RO removal of these pathogens is based on size exclusion.

Table 1-1. Log Reduction Attained by GWRS Treatment Process.

Pathogen (Log Reduction Required)	WWTP	MF + Cl_2	RO	UV/ AOP	Underground Retention Time	Total
Giardia (10)	0	4+	2+	6	0	12+
Cryptosporidium (10)	0	4+	2+	6	0	12+
Viruses (12)	0	0	2+	6	4	12+

Notes: MF + Cl_2 = microfiltration which includes chlorine addition ahead of MF to form chloramines; RO = reverse osmosis; UV/AOP = ultraviolet light / advanced oxidation process; WWTP = wastewater treatment plant; "+" indicates that actual log reduction demonstrated may be (slightly) higher based on monitoring (daily pressure decay test for MF and online TOC monitoring for RO).

1.4 Broader Benefits and Rationale for Study

A greater number of demonstrated, credited virus LRVs for potable reuse would have multiple benefits including improving regulatory and public confidence in the potable reuse process, achieving excess credits above requirements toward a greater safety factor, and reducing the

required underground travel time (for indirect potable reuse, IPR) for the purposes of pathogen control. Reducing required underground travel time has notable project design and siting benefits since it increases the number of viable injection, recharge, and potable extraction sites for groundwater augmentation projects.

More broadly, the California SWRCB recommended, through work with an Expert Panel, specific research to address certain knowledge gaps in direct potable reuse (DPR) to enhance understanding and acceptability of DPR in California (Olivieri et al. 2016). The recommended (and now completed) research included measurement of pathogens in untreated (raw) wastewater to improve the water reuse industry's understanding of pathogen concentration in raw wastewater, as well as exploring approaches for determining pathogen log removal values (LRVs) and probabilistic risk modeling. Thus, including and extending beyond California, the research completed in the present study compliments these efforts and has national and worldwide value for other regions' potable reuse efforts, benefiting the design and management of both IPR and DPR projects.

Both IPR and DPR projects must meet certain pathogen removal requirements to ensure public health. For IPR projects as noted previously, demonstrating additional LRVs may reduce the required underground travel time, which serves as an "environmental buffer" (storage of advanced treated water underground or in a surface water reservoir, providing additional treatment). This buffer is absent from DPR schemes. Enhanced pathogen LRV credit is particularly needed for DPR projects because they do not achieve credits from underground retention while at the same time will be required to demonstrate greater pathogen log removal than required for IPR. Thus, for DPR projects, enhanced LRV crediting of existing processes (such as wastewater treatment and RO) may reduce the overall required number of engineered unit processes during advanced treatment for the purposes of pathogen control. It could also reduce costs where additional engineered treatment would otherwise be needed. Improved strategies for demonstrating and crediting pathogen removal are needed more than ever as utilities and water providers increasingly consider potable reuse projects to address water security due to regional water scarcity and growing demand.

CHAPTER 2

Background and Site Description for Wastewater Treatment Evaluation of Virus Removal

Potable reuse is the recycling of (waste)water to create a drinking water supply via advanced purification. Raw wastewater from the sewer collection system is first treated at a wastewater treatment plant (WWTP). WWTPs are traditionally designed to treat the raw wastewater to a secondary or tertiary standard so that it is safe to return to the environment and/or to be used for non-drinking recycled water applications (i.e., non-potable reuse). In the case of potable reuse, all or some of the secondary or tertiary treated wastewater is instead sent to a water purification facility such as the GWRS AWPF for advanced treatment to meet drinking water standards.

To determine virus removal during wastewater treatment toward a potential log credit for GWRS, OCWD conducted a study to evaluate enteric virus concentrations at the OC San Reclamation Plant No. 1 (P1) and Treatment Plant No. 2 (P2) wastewater treatment facilities. Broadly, the study primarily addressed how enteric virus removal at both wastewater treatment plants can be demonstrated using a 24-sampling event monitoring campaign, and how the results of this campaign can be used to develop virus-specific log removal values (LRVs) for regulatory crediting. This work serves as a case study for demonstrating log removal at a WWTP.

The specific objectives of the study were to:

- determine the concentration and variability of enteric viruses, male-specific (MS) and somatic (SOM) coliphage, and total and fecal coliform microbial indicators in OC San's raw wastewater influent at both P1 and P2 and in the following four secondary effluents: OC San P1 activated sludge (AS) effluents (AS1 and AS2), P1 trickling filter (TF) effluent, and P2 trickling filter/solids contactor (TF/SC) effluent;
- determine the associated LRV for each process (P1 AS1, P1 AS2, P1 TF, and P2 TF/SC) using lognormal statistical modeling techniques including a covariance-based analysis and a Monte Carlo simulation to calculate probability distributions; and,
- determine a single, conservative log removal credit value to propose to regulators based on the observed LRVs for each process, along with P1 and P2 operating range values (ORVs) on which any credited LRV would be contingent.

The study approach built on a successful pathogen LRV study previously completed at City of San Diego's North City Water Reclamation Facility (Trussell Technologies 2017a). An initial test plan for the OC San study describing the proposed approach was prepared by OCWD staff and submitted to the California SWRCB DDW for review; DDW comments were incorporated into OCWD's final test plan (OCWD 2019) and DDW approved the test plan in June 2019 (SWRCB DDW 2019). Comments from the Microbiological Subcommittee of the OCWD GWRS Independent Advisory Panel (IAP) were also incorporated (NWRI 2018). Key recommendations

of the GWRS IAP subcommittee on the test plan included that OCWD complete certain planned method development work related to virus sampling and recoveries for tested wastewater matrices prior to finalizing the test plan and beginning monthly sampling. OCWD completed this work in collaboration with the project laboratory partner, Michigan State University (MSU), and included those findings in the final approved test plan, along with other IAP comments and recommendations.

As the project progressed, the full OCWD GWRS IAP, including the members of the Microbiological Subcommittee, convened in October 2020 to provide comments and feedback on study findings to date (at that time, included data from May 2019 through March 2020). Feedback from the IAP included considerations for the use of molecular assay data and review of OCWD's potential approach for determining a single, consolidated LRV from the four blended OC San secondary effluents. The IAP also agreed with OCWD's recommendation to extend the study sampling into Spring 2021 related to project interruption that had occurred related to the coronavirus disease pandemic (COVID-19) in order to ensure all four seasons had sampling coverage (NWRI 2020).

A study report dated June 25, 2021, was provided to DDW for their review as part of the Title 22 Engineering Report Addendum No. 2 for the GWRS Final Expansion (Title 22 ER Addendum 2) as well as to the IAP for their October 2021 review. DDW indicated a preference to avoid calculating the consolidated LRV credit as a flow-weighted average for the four separately determined LRVs (i.e., flow-weighted average of the 5th percentile of each process's calculated removal in percent removal form, subsequently converting the flow-weighted average to log removal). Feedback from the IAP after the 2021 review supported this recommendation as well as indicated that the modified Monte Carlo simulation analysis for determining 5th percentile LRV (presented later in this report), while preferable to a standard Monte Carlo approach, was not as suitable for the OC San datasets and that a covariance-based analysis was instead preferable. As a result, a single 5th percentile LRV taken from the single lowest performing wastewater treatment process was proposed as virus credit. A detailed analysis of the approach, including a comparison of both statistical analyses, are detailed in this report.

2.1 OC San Facilities Description

OC San is a resource recovery agency that collects, treats, disposes, and recycles wastewater that is generated by 2.6 million people in Central and Northern Orange County, California. Two (2) treatment plants are operated and maintained by OC San. Additionally, OC San maintains 15 offsite pump stations and 386 miles of sewers. As a resource recovery facility, OC San converts sewage into water, energy and agricultural fertilizers. A portion of the organics in the incoming sewage are converted to energy in the form of electricity, process heat, and building cooling. The organics not converted to energy are converted to biosolids and are used for their nutrient-rich benefits to fertilize farmland and as compost for agriculture (OC San 2015).

The two separate WWTPs operated by OC San are Reclamation Plant No. 1 (P1) in Fountain Valley and Treatment Plant No. 2 (P2) in Huntington Beach. OCWD currently receives essentially all OC San P1 effluent and recycles it either via advanced treatment for potable use as part of the GWRS or via tertiary treatment for non-potable use. The P2 effluent is currently not

recycled by GWRS, but the majority will be upon completion of the GWRS Final Expansion (GWRSFE) project in 2023. Figure 1-1 (see Chapter 1) shows the locations of the GWRS facility, and OC San's two treatment plants.

2.2 OC San Treatment Processes and Sampling Locations

Simplified flow diagrams for the OC San P1 and P2 treatment processes are illustrated below in Figure 2-1 and Figure 2-2, respectively. Sampling locations showing where raw wastewater and secondary effluent samples were collected for this study along each treatment train are depicted. Raw wastewater influent samples and secondary effluent samples were collected from both OC San P1 and P2 to determine the overall removal of specific microbial indicators and enteric virus targets.

Raw wastewater entering OC San P1 is treated through preliminary screening and primary clarification with chemical addition and is then diverted into one of two secondary treatment trains that operate in parallel (trickling filter process or activated sludge process). Secondary treated water from OC San P1 is ultimately delivered to OCWD for advanced purification as part of GWRS. For this study, raw wastewater from P1 was collected after primary bar-screening but before primary clarification and chemical addition. The GWRS AWPF microfiltration backwash waste (BWW) stream enters P1 primary effluent prior to secondary treatment by the AS process, at Primary Clarifiers 6-31.

Secondary effluents generated by each of three parallel treatment processes at P1 were sampled in this study. The first P1 treatment train routes the primary effluent through a trickling filter (TF) process followed by secondary clarification. Treated effluent from the TF process was sampled and is represented in Figure 2-1 by sampling location 2. The other train sends wastewater through two parallel trains of the activated sludge (AS) process, designated separately as AS1 and AS2. Secondary effluent samples taken from each AS process following secondary clarification are shown in Figure 2-1 as sampling locations 3 and 4 for AS1 and AS2, respectively. Both AS trains operate in the nitrification-partial denitrification (NDN) mode. The major difference between the AS1 and AS2 processes is that AS1 does not receive mixed liquor return, while the newer AS2 facility does receive it. To sample AS2 effluent for the present study, effluent from AS2 West and AS2 East clarifiers were sampled separately and mixed manually on site proportionally to the plant-measured instantaneous flows to serve as the AS2 effluent sample.

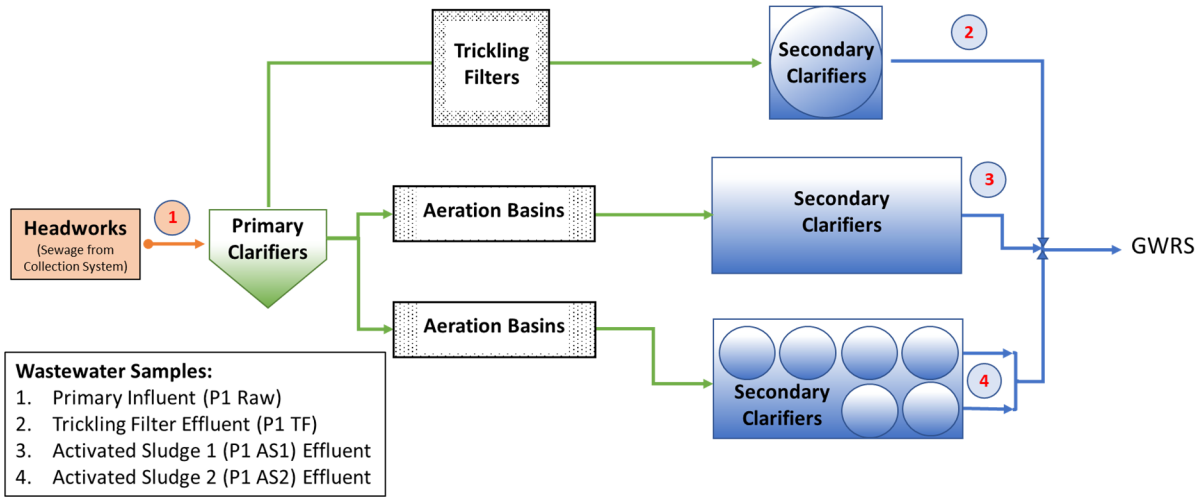


Figure 2-1. Simplified Flow Diagram Illustrating Two Parallel Secondary Treatment Trains at OC San's Reclamation Plant No. 1 and the Study Sampling Locations.

Plant secondary clarifiers that follow TF, AS1, and AS2 have engineering differences illustrated simplistically in the diagram.

From P1, GWRS currently receives a blend of AS1, AS2, and TF effluents. Excess daytime AS2 secondary effluent is stored using OCWD secondary effluent flow equalization (SEFE) tanks and then used to supplement GWRS feedwater supplies during the late evening and early morning in order to maintain a constant influent flow rate to GWRS despite the normal diurnal variation in WWTP effluent flows.

OC San P2 treats wastewater from the western and coastal parts of the OC San service area along with the Santa Ana Regional Interceptor (SARI) trunkline (brine line), the centrate flows from thickening and dewatering of biosolids from P1 and P2, and other side stream flows. After preliminary screening and primary clarification, wastewater is treated by one of two secondary treatment trains that operate in parallel: the high purity oxygen activated sludge (HPOAS) plant and the trickling filter/solids contactor (TF/SC) process, the latter of which is illustrated in Figure 2-2. For the present study, raw wastewater for P2 was collected after preliminary screening but before chemical addition, primary clarification, and centrate return flows. The effluent from both secondary treatment trains is currently discharged into the ocean after blending with unreclaimed effluent from P1. As part of the GWRSFE project, the P2 SARI trunkline and select side streams will be segregated at the P2 headworks and routed for treatment through the HPOAS process, and the HPOAS treated effluent will continue to be discharged to the ocean. After segregation, effluent from only the P2 TF/SC process will be conveyed to GWRS to supply the expanded AWPF. Therefore, secondary effluent samples from P2 for the present study were collected only for TF/SC treated effluent, following secondary settling clarifiers as shown in Figure 2-2 sampling location 2.

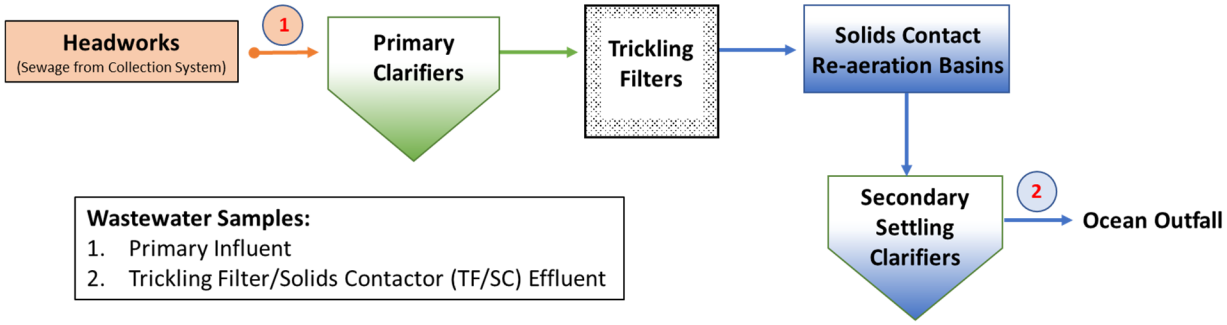


Figure 2-2. Simplified Flow Diagram Illustrating the Trickling Filter/Solids Contactor (TF/SC) Train at OC San's Treatment Plant No. 2 and the Study Sampling Locations.

In summary, a total of six sample locations were included in the study from OC San P1 and P2. Sampling locations for OC San P1 included raw wastewater influent, TF secondary effluent, AS1 secondary effluent, and AS2 secondary effluent, while sampling locations from OC San P2 include raw wastewater influent and TF/SC secondary effluent. Microbial concentrations of both the AS1 and AS2 effluent streams from P1 were of interest due to the operational differences between the two processes described above. Meanwhile, sampling at P2 was limited to characterizing the TF/SC process since only P1's TF/SC secondary effluent will be supplied to GWRS in the future as part of the GWRSFE project.

CHAPTER 3

Microbial Monitoring and Data Collection

To evaluate enteric virus removal from the OC San wastewater treatment facilities (P1 and P2), grab samples and ultrafilter-concentrated samples were collected from raw wastewater and secondary effluent sampling locations. The sampling locations are described previously in Section 2.2. Prior to initiating the 24-sampling event monitoring schedule, a method optimization study was conducted to determine the best methodological approach for enteric virus and coliphage detection and recovery, including confirming sufficient sample volume required to ensure detection. The method of enumeration for each microbial target monitored and their respective corrections based on method recoveries are described in detail below.

3.1 Microbial Targets

At the sampling locations described in Section 2.2, microbial targets were monitored by collection of OC San P1 and P2 raw wastewater and secondary effluent. Microbial targets evaluated in this study are listed in Table 3-1 and include male-specific (MS) and somatic (SOM) coliphage, total and fecal coliform, and enteric viruses:

- MS and SOM coliphage were enumerated for both raw wastewater and secondary effluent samples using EPA Method 1602 with double agar layer modification (EPA 2001). Data are reported as plaque forming units per liter (PFU/L).
- Total and fecal coliform were enumerated using standard methods 9222B and 9222D, respectively, and are reported as colonies per liter (SM 2018).
- Enteric viruses were evaluated according to the EPA 1615 standard method, which describes the cultivable and molecular detection methods (EPA 2010).
    - The cultivable virus (infectivity) assay utilizes the Buffalo Green Monkey (BGM) kidney cell line to produce cytopathic effects (CPE) in replicate culture flasks to enumerate the concentration of infectious enteric viruses. The number of flasks demonstrating CPE are then used to quantify the most probable number (MPN) of infectious units per liter of environmental sample using EPA's MPN calculator (EPA 2010).
    - For molecular detection of enteric viruses under EPA 1615, a modified molecular detection method using droplet digital polymerase chain reaction (ddPCR) was utilized in this study to quantify the amount of genetic material present within a sample. The targeted enteric viruses were enterovirus and norovirus GII, which are reported as gene copies per liter (GC/L) and may include both infectious virus particles and inactivated virus material. For each virus target, fluorescence-positive or fluorescence-negative droplets were counted after PCR to calculate gene copies per liter volume using Poisson statistics.

Table 3-1. Microbial Targets Included in OC San Virus LRV Study.

Constituent	Analytical Method	Laboratory	Rationale for Testing
Enteric viruses	EPA 1615 infectivity culture assay	BCS	Regulated pathogen; characterize concentrations and LRVs through wastewater treatment processes
Enterovirus and Norovirus GII	EPA 1615 modified ddPCR[1]	MSU	Research data to complement enteric virus culture assay[2]; characterize gene copy count and LRVs through wastewater treatment processes
Male-specific (MS) and somatic (SOM) coliphage	MS and SOM coliphage[3] by EPA 1602	MSU	Enteric virus surrogate in wastewater; determine virus recovery efficiency; characterize concentrations and LRVs through wastewater treatment processes
Total coliform	Membrane Filter (MF) 9222B	OC San	Regulated pathogen and indicator organism; characterize concentrations and LRVs through wastewater treatment processes
Fecal coliform	Membrane Filter (MF) 9222D	OC San	

Notes: BCS = Biological Consulting Services of North Florida. MSU = Michigan State University (MSU) Water Quality and Environmental Microbiology Laboratory.

[1] Enteric virus analysis with a modified ddPCR assay.

[2] After collection, each environmental sample was split for analysis for both the cultivable assay and the molecular assay. Raw influent grab samples were split after PEG concentration while secondary effluent ultrafilter samples were split after ultrafilter elution.

[3] EPA 1602 with double agar overlay modification. Modification involves the addition of a portion of the environmental sample to melted agar culture media containing host *E. coli* bacteria and pouring this mixture onto a hard layer of agar media to count formation of plaques. Growth and spread of cultivated coliphage is restricted by the agar gel to accurately estimate plaque formation for both raw wastewater and secondary effluent samples.

3.2 Sample Collection

After performing a method optimization study, the study team found that polyethylene glycol (PEG) concentration steps for raw wastewater samples were recommended over direct inoculation because an overall higher number of enteric viruses were detected using the PEG method and a larger more representative sample can be analyzed despite increased handling time that may lead to virus loss. Furthermore, optimization of virus recovery experiments determined that raw wastewater matrix spike recovery (MSR) experiments processed by PEG concentration for enteric viruses, somatic and MS coliphage detection were within acceptance criteria for groundwater for standard method 1615 and 1602, respectively (EPA 2001, EPA 2010). For this reason, the project team determined that large volume concentration of raw wastewater was not necessary.

With respect to secondary effluent, matrix spike recoveries for ultrafiltered secondary effluent showed a lower than acceptable recovery for somatic coliphage (17%). Direct testing (i.e., no ultrafiltration) of somatic and MS coliphage for secondary effluent samples showed a greater recovery within EPA 1602 acceptance criteria, and it was therefore recommended that coliphage enumeration be performed on sample grabs (EPA 2001). Since recovery of enteric viruses by PEG concentration of secondary effluent grab samples was poor, larger collection volumes via onsite ultrafiltration are required for detection of enteric viruses from secondary

effluent. From this preliminary work, an optimal set of sample volumes and extraction technique for each sample type and method were determined and are summarized below in Table 3-2.

Table 3-2. Summary of Sample Volume and Type for Coliphage and Enteric Virus Analysis at OC San P1 and P2.

Site name	Sample volume	Type of sample	Analysis
OC San Plant No. 1			
RWW	1500 mL	grab sample	EPA 1602 (coliphage); EPA 1615 PEG (virus – culture assay and ddPCR)
TF	1500 mL	grab sample	EPA 1602 (coliphage)
	25 liters	concentrate sample on UF filter	EPA 1615 pressurized PBS (virus – culture assay and ddPCR)
AS1	1500 mL	grab sample	EPA 1602 (coliphage)
	25 liters	concentrate sample on UF filter	EPA 1615 pressurized PBS (virus – culture assay and ddPCR)
AS2	1500 mL	grab sample	EPA 1602 (coliphage)
	25 liters	concentrate sample on UF filter	EPA 1615 pressurized PBS (virus – culture assay and ddPCR)
OC San Plant No. 2			
RWW	1500 mL	grab sample	EPA 1602 (coliphage); EPA 1615 PEG (virus – culture assay and ddPCR)
TF/SC effluent	1500 mL	grab sample	EPA 1602 (coliphage)
	25 liters	concentrate sample on UF filter	EPA 1602 (coliphage); EPA 1615 pressurized PBS (virus – culture assay and ddPCR)

Notes: RWW = Raw Wastewater; TF = trickling filter secondary effluent; AS1 = activated sludge effluent from train 1; AS2 = activated sludge effluent from train 2; TF/SC = trickling filter solids contact; UF = ultrafiltration; PEG = polyethylene glycol; PBS = phosphate buffered saline; ddPCR = droplet digital polymerase chain reaction

Grab samples were collected in one liter sterile-autoclaved high-density polyethylene bottles. To collect a grab sample, the sample tap was flushed for approximately 2-3 minutes before carefully placing the collection bottle underneath the sample tap, ensuring no physical contact was made between the bottle mouth and any surface. Immediately after collection, the sampling time, date, and location were written on the sample label and immediately placed on ice.

For collection of secondary effluent samples, a hollow-fiber ultrafilter tubing assembly was used to capture 25 liters of secondary effluent. All tubing, fittings, and caps were sanitized and autoclaved in a controlled laboratory setting prior to use. Sterilized equipment was unpacked on-site and assembled by OCWD staff. To concentrate OC San secondary effluent, the hollow-fiber ultrafiltration assembly was set up as described by the manufacturer's instructions with a single-use dead-end hollow fiber ultrafiltration cell. All ultrafilter cells (Rexeed-25 S filter kit by Innovaprep) were prepared at each sampling location using the same procedure as follows: the blue end of the ultrafilter cell, which houses the hollow fibers, was used to mark the feed port (end receiving OC San sampled water), while the red end of the filter cell was set as the

permeate/retentate port (drain). A pressure gauge and peristaltic pump was installed onto the feed tubing assembly preceding the hollow fiber filter to pump and monitor inlet water pressure. To complete the assembly, a downstream flow meter was installed to the permeate tubing to monitor the volume of water sampled.

Upon filtration of 25 liters (at a rate of 1 liter per minute), the peristaltic pump was turned off and the inlet tube line was removed from the sample tap. Any remaining water within the tubing assembly was removed by ensuring all pinch-clamps are placed in the open position and inserting a sterile 2 µm cartridge filter on the inlet tube to avoid aerosol contamination of intake. With the peristaltic pump switched on for approximately 1 minute, the remaining void water was purged from the tubing assembly. Prior to disassembly, sample time, date and location were written on the sample label. Ultrafilter cells were then immediately and carefully unmounted, re-capped, wiped and preserved on ice.

Upon arrival at MSU, filter cell concentrates were extracted from each ultrafilter cell using a high-volume elution canister consisting of pressurized 0.075% Tween 20 PBS. Using a sanitized canister adapter fitting, the sample is eluted by manual de-pressurization of the canister with the ultrafilter cell retentate port (drain) open, and the filtrate port (permeate port) closed. An approximate volume of 100 - 120 mL is eluted off the filter and immediately passed through a 0.45 µm filter to remove large debris and bacterial cells.

After processing of both raw wastewater and secondary effluent samples, each sample was divided into a series of subsamples by the Water Quality and Environmental Microbiology Laboratory at MSU to protect samples from multiple freeze-thaw cycles. For sample analysis, one subsample is used for culture-based enteric virus analysis, and one subsample is used for DNA extraction, reverse transcription (for RNA virus targets), and subjected to polymerase chain reaction (PCR) to quantify the viral particle concentration. The last subsample is stored for archival purposes, which ultimately proved quite useful in this study since enteric virus samples previously analyzed by MSU were required to be re-analyzed by Biological Consulting Services of North Florida (BCS). Archived subsamples were required to address MSU laboratory impacts described in the next section and to ensure that all enteric virus data used to calculate log removal credit was generated by one laboratory for consistency.

3.2.1 Impact of the COVID-19 Pandemic

The planned 24 sampling events began in late May 2019 and were scheduled to be completed in approximately one year at a rate of two events per month. However, due to the outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulting in the COVID-19 pandemic, sample collection from OC San's P1 and P2 sampling sites was suspended during the period of March 2020 through mid-October 2020. Suspension of sample collection was due to the closure of the project laboratory partner MSU Water Quality and Environmental Microbiology Laboratory, mandated by the state of Michigan. Sample collection resumed in late October 2020 to achieve the originally planned total of 24 sampling events. The unanticipated delay in sample collection resulted in a non-continuous dataset. If the two event per month sampling schedule had continued at that rate after resumption of sampling, sample collection could have been completed in Winter 2020. However, to avoid a seasonal gap with no data

representing a spring season, the study sampling schedule was extended into Spring 2021 such that the 24 sampling events are approximately uniformly distributed throughout the seasons of the calendar year. As a result, the project's final sampling event occurred in April 2021. Accordingly, all calendar months were sampled at least once, with most months sampled twice (and some three times), and one month having been sampled four times.

As an additional impact of the COVID-19 pandemic, all cultivable virus analysis was completed by Biological Consulting Services of North Florida (BCS) rather than the originally planned MSU Water Quality and Environmental Microbiology Laboratory noted in the test plan (OCWD 2019). After temporary closure of the MSU laboratory, MSU staff recommended that all cultivable virus samples be analyzed by BCS due to the limited capacity of the MSU laboratory and sample backlog related to delays brought on by the COVID-19 pandemic. Based on a recommendation from the MSU laboratory, BCS was chosen due to its strong performance record and high throughput analysis capability to process archived cultivable virus samples from the study that had previously been analyzed by MSU, as well as to analyze the remainder of the study's planned cultivable virus samples. As indicated in Table 3-1, the other microbial targets were analyzed by MSU and OC San as originally planned.

3.3 Sampling Frequency

A total number of 24 sampling events over the course of one year was originally scheduled but required more than the one year to complete due to delays related to the nationwide SARS-CoV-2 pandemic as noted above. A total of 24 events was planned based on experience from the San Diego Pure Water Project study (Trussell Technologies 2017a) and expected regulator preference for at least 24 events.

Microbial sampling at OC San P1 and P2 began on May 28, 2019, and, prior to the COVID-19 pandemic, continued at a frequency of two (2) sampling events per month. Each sampling event, which spanned between one and three days, included samples from all six locations (four sampling locations at P1 and two sampling locations at P2 as described above in Section 2.2) for enteric virus and coliform enumeration. Duplicate samples were collected for all microbial targets at least twice for all sites on a staggered schedule except for P2 raw influent which was collected once over the course of the study. Grab samples for coliphage evaluation were collected once a month from all six locations contemporaneously with enteric virus samples, such that a total of 12 coliphage sampling events were reported over the study.

During sampling, no attempt was made to time sample collection of effluent versus influent according to the average system hydraulic residence time, i.e., Lagrangian sampling, since removal of virus would be calculated based on a statistical approach using the overall study observed distribution of influent and effluent concentrations (see next chapter). Such a statistical approach is considered superior to same day influent-effluent pairing (Trussell Technologies 2017b).

Upon recommencement of sample collection in late October 2020 after delays brought on by the COVID-19 pandemic, sampling events were scheduled at typically two sampling events a month, with some events occurring only once per month related to the extension of the final

sampling events into Spring 2021. The distribution of sampling dates for each microbial target is illustrated in Table 3-3 below. Periodic make up events, denoted as event 'M' below, were required to meet sample acceptance criteria when the original samples were not received on time due to shipping issues (e.g., shipping company was occasionally unable to deliver samples in time to meet 24-hour hold time for grab samples of raw influent and 72-hour hold time for secondary effluent samples obtained with an ultrafilter). Note that these make up events did not necessarily include sample collection from all sampling locations, only the locations that missed hold times in prior sampling events.

Table 3-3. Sampling Event Summary for Each Microbial Target.

	2019															2020										2021					
	May	Jun		Jul		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar		Oct	Nov		Dec	Jan		Mar		Apr	
Event Number:	1	2	3	4	M	5	6	7	M	8	9	10	11	12	13	14	15	16	17	18	☀	19	20	21	M	M	M	M	22	23	24
Enteric Virus (Total Cultivable Virus Assay)																														
Enterovirus/Norovirus GII (Molecular Detection Assay)																														
Total and Fecal Coliform																														
Event Number:	1	2			3				4		5		6		7		8			☀		9	10		11			12		
SOM and MS Coliphage																														

M Make-up event
No sample collected
Sample collected
☀ Sampling postponed due to COVID-19

3.4 Method Recovery for Virus Concentration Corrections

To determine virus recovery efficiency, matric spike recovery (MSR) samples were collected contemporaneously with a subset of the enteric virus and coliphage samples. A total of 9 raw wastewater matrix spike recovery samples were collected per raw wastewater sampling location over the 24 total samples (i.e., greater than approximately one in three raw wastewater samples featured a paired matrix spike recovery sample), which is a slightly greater number than planned per the regulator-approved test plan (OCWD 2019). For each secondary effluent sampling location, five matrix spike recovery samples were collected, which given four secondary effluent sampling locations resulted in 20 total secondary effluent matrix spike recovery samples over the course of the study (i.e., greater than approximately one in five secondary effluent samples [20 of 96] featured a paired matrix spike recovery sample). The lower number of overall matrix spike recovery sampling for secondary effluent location compared to raw wastewater was deemed appropriate for the less challenging secondary effluent matrix.

For cultivable enteric virus and MS and SOM coliphage, native concentrations were corrected by the measured method recovery. Method recovery is estimated by calculating the percentage of virus recovery obtained from matrix spike sample results. For example, correcting for a 50% recovery measurement (i.e., half of the known concentration of spiked virus target observed) will double the reported native concentration. Percent recovery results are then used to calculate an accurate (corrected) native virus concentration.

Specifically, native cultivable virus concentration data were corrected using the average cultivable recovery of MS coliphage, SOM coliphage, and poliovirus. Recovery data from all three targets were pooled to calculate a single average recovery value which was then used to correct the native results. Coliphage concentration data were corrected using the average cultivable recovery of MS coliphage and SOM coliphage only. These targets of known concentration were spiked into raw influent samples directly and into the ultrafiltration unit for secondary effluent samples by the MSU laboratory, for P1 and P2 wastewater split samples specifically collected for the purpose of matrix spike recovery determination for a subset of sampling events over the course of the study.

Due to matrix differences (raw wastewater and secondary effluent matrices), recovery corrections were calculated separately for P1 raw influent, P2 raw influent, P1 secondary effluent and P2 secondary effluent each using their respective corresponding group of matrix spike samples. For P1 secondary effluent, recovery measurements were pooled and averaged for all three P1 secondary treatment processes (i.e., P1 AS1, AS2, TF) based on similar ranges of recoveries. A summary of microbial target recoveries is shown below in Table 3-4.

Table 3-4. Summary of Percent Recoveries for All Virus Targets.

OC San Sampling Site		EPA 1602[1]		EPA 1615[2, 3]		
		SOM Coliphage	MS Coliphage	Poliovirus	Enterovirus	Norovirus GII
Plant No. 1 Raw Influent	Range (n)	75-163% (7)	34–116% (6)	16–363% (9)	0–6547% (9)	0 – 6579% (9)
	Median	113%	63%	47%	44%	0%
	Average	**120%**	**69%**	103% (**99%**)	841%	743%
Plant No. 1 Secondary Effluent	Range (n)	59–130% (10)	13–118% (11)	4 – 68% (15)	0.1 -176% (15)	0 – 174% (15)
	Median	86%	96%	21%	2.9%	0.2%
	Average	**88%**	**76%**	24% (**58%**)	18.2%	12.9%
Plant No. 2 Raw Influent	Range (n)	60–113% (7)	36-156% (7)	31–613% (9)	24 – 964% (9)	0 – 953% (9)
	Median	79%	81%	67%	45%	5.0%
	Average	**83%**	**86%**	132% (**103%**)	223%	122%
Plant No. 2 Secondary Effluent	Range (n)	69%-149% (4)	50–114% (4)	8-63% (5)	0.4 – 87% (5)	0 – 87% (5)
	Median	100%	71%	21%	9.0%	0.1%
	Average	**104%**	**76%**	27% (**66%**)	22%	19.3%

Notes:

1. Average recovery percentages shown in bold were used to correct each respective native dataset, e.g., 120% recovery was used to correct all native P1 raw influent samples for SOM coliphage.
2. The average percent recovery used to correct the native cultivable virus dataset was obtained by taking an average of SOM, MS and Poliovirus recoveries, which are shown as the bold mean values for Poliovirus.
3. An average recovery value was not used to correct the native molecular (enterovirus and norovirus GII) datasets.

For the molecular assay (ddPCR), virus recovery was measured from matrix spike samples by spiking armored RNA (which consists of a gene fragment of the virus target at a known concentration), which is used as an inhibition control in EPA 1615 method, to samples as described above for cultivable virus and coliphage. Recovery of enterovirus and norovirus GII armored RNA indicated inconsistencies for the enterovirus recovery hence only norovirus GII recovery results were considered for native sample correction. However, norovirus GII recoveries were highly variable ranging from frequent instances of zero percent recovery to up

to 180% for raw wastewater and 20% for secondary effluent, with a limited number of extremely high outliers observed for raw wastewater (e.g., 6580%). Instances of measured negative recovery (i.e., spiked result lower than before spike) where converted to zero recovery in Table 3-4. Reasons for the wide recovery performance are unclear but may be related to potential incompatibility of use of the ddPCR analysis with armored RNA targets (which are traditionally used as an inhibition control in EPA 1615 method using qPCR). Therefore, uncorrected native ddPCR data was used in this study for determining enterovirus and norovirus GII log removal.

3.5 Plant Performance Monitoring during Enteric Virus Sampling Period

Regular P1 and P2 operational performance data collected by OC San as part of routine plant monitoring were analyzed during the LRV study sampling period to develop an understanding of the operational envelope representative of normal operations and conditions at OC San P1 and P2. OC San operational parameter and water quality data were acquired with assistance from OC San operations engineers. Many key parameters such as influent and effluent flow rates and turbidity are monitored continuously by online instruments. Other key parameters are monitored via regular grab or composite samples such as for biological oxygen demand measurements.

In consultation with OC San staff, the performance data from P1 and P2 were reviewed and analyzed using statistical methods to develop recommended OC San operating range values (ORVs) describing the normal operating envelope to be associated with any LRV GWRS virus credit. Details of this analysis and the resultant ORVs are provided in Chapter 5.

CHAPTER 4

Determination of Wastewater Treatment Log Removal Using a Statistical Approach

This section describes two mathematical approaches used for calculating LRVs of enteric viruses and coliphage microbial targets for each secondary treatment process. If a wastewater treatment facility has one treatment process that produces treated effluent as source water for potable reuse, its conservative LRV (e.g., 5th percentile LRV), may be proposed for LRV credit. In the case of OCWD GWRS, four parallel treatment processes produce secondary effluent that blends to provide the source water for potable reuse. To determine the appropriate LRV to propose, 5th percentile LRVs for each treatment process were calculated using either a covariance-based approach or Monte Carlo simulation with microbial concentration data obtained from the 24 sampling events for each treatment process.

The key difference between these two approaches is that the covariance-based approach assumes that the influent concentration distribution and effluent concentration distribution are dependent, whereas the Monte Carlo simulation approach assumes that these two distributions are independent. Two events are considered statistically independent if the occurrence of one does not affect the probability of occurrence of the other. Both approaches are based on established methods for calculating the difference between two microbial concentration datasets that are lognormally distributed. Although the original intent when developing the present study sampling design (test plan) was to perform a Monte Carlo-based statistical analysis to derive the calculated LRV, the potential suitability of the alternative covariance-based approach was later suggested by the GWRS Independent Advisory Panel (IAP); both approaches can be performed with the sampling design used in this study, as described in Chapter 3. Whereas the covariance-based approach is calculated directly using the equation described below in Section 4.1, the Monte Carlo simulation approach (Section 4.2) represents an estimate of an analytical solution that could alternatively be directly used. This chapter describes how results were obtained for each statistical model with the goal to determine the most appropriate conservative GWRS log removal credit value.

4.1 Covariance Approach to Determine Log Removal

The present study applied a probabilistic approach that uses covariance analysis, herein referred to as the covariance approach. An abstractly similar but more simplified approach is described in Trussell Technologies (2017a) as a parametric approach and is illustrated in Figure 4-1 below. In this very simplified approach, the microbial target concentrations for the constituent of interest are plotted on a logarithmic probability distribution. When plotted this way, the difference (gap) between the raw wastewater influent compared to secondary effluent concentration distributions represents the observed log removal, i.e., 1-log removal in this example (Figure 4-1). Depending on how parallel the two distributions are, this difference (gap) may be consistent along the distribution, or it may be narrower at the low or high end, as

described by Trussell Technologies (2017a). Thus, a conservative estimate of LRV corresponds to the smallest difference between the two distributions.

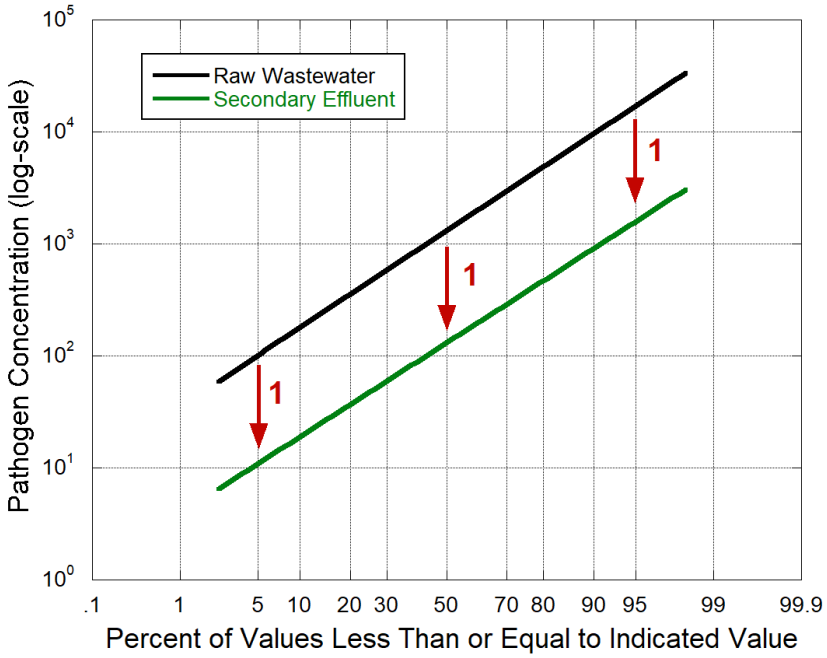


Figure 4-1. Theoretical Example LRV Determined from Parametric Approach.

In contrast, the covariance analysis approach as described by Tchobanoglous et al. (2021) is based on the sample means for influent and effluent and incorporates sample dataset covariance to enable calculation of the 5th percentile (or any percentile). With this approach, influent and effluent data are assumed to be dependent (correlated). The approach uses an estimate of sample means and standard deviations of two correlated random normal distributions, namely, the raw influent and effluent distributions. In the present study, microbial concentration data were analyzed by this approach using Microsoft Excel software (see Appendix A for Covariance Tool).

To calculate LRVs for each treatment process using the covariance approach, each sample concentration result within the influent and effluent distributions is first log-transformed. The average LRV (μ_{LRV}) is calculated as shown in Equation 4-1:

$$\mu_{LRV} = \mu_{Inf} - \mu_{Eff} \qquad \text{(Equation 4-1)}$$

where: μ_{Inf} = the average log-value for the influent distribution.

μ_{Eff} = the average log-value for the effluent distribution.

To determine covariance, the log-transformed influent and effluent data are sorted from smallest to largest. These ranked values from the influent distribution are paired with ranked values from the effluent distribution (i.e., rank paired) prior to calculating sample covariance. Note that to determine if two distributions are covariant, the number of entries from each distribution must be the same, that is, the number of influent samples must equal the number

of effluent samples. Equation 4-2 is then used to estimate the LRV probability distribution using the average LRV term calculated in Equation 4-1, the z-score statistic, and the standard deviation of sample covariance:

$$\text{LRV} = \mu_{LRV} + (Z)\sigma_{var\text{-}\Delta} \qquad \text{(Equation 4-2)}$$

where: μ_{LRV} = the average LRV calculated in Equation 4-1.

Z = z-score, or standard score for a given probability such as the 5th percentile.

$\sigma_{var\text{-}\Delta}$ = the standard deviation of sample covariance between the influent and effluent distributions.

The standard deviation of sample covariance ($\sigma_{var\text{-}\Delta}$) is calculated using Equation 4-3, below, which requires solving for the variance of the influent distribution, variance of the effluent distribution, and the covariance between both influent and effluent distributions. These parameters can be calculated using Microsoft Excel's variance and covariance.s formulas, respectively, for each respective log-transformed distribution.

$$\sigma_{var\text{-}\Delta} = \sqrt{[var(Inf) + var(Eff) - 2cov(Inf, Eff)]} \qquad \text{(Equation 4-3)}$$

where: *var(Inf)* = variance of the influent distribution.

var(Eff) = variance of the effluent distribution

cov(Inf,Eff) = the covariance correlation between both the influent and effluent distributions.

The 5th percentile LRV is obtained by using the 5th percentile z-score, or z = (-) 1.645, in Equation 4-2. LRV probability plots for each treatment process and microbial target were generated using the 5th, 50th, and 95th percentile LRVs using Equation 4-2. Final LRV results using this approach are described in detail in Chapter 5 and a complete summary table of the results can be found in Section 5.3.

4.2 Monte Carlo Simulation Approach to Determine Log Removal

The second statistical approach used to calculate median and 5th percentile LRVs for each treatment process was the Monte Carlo simulation approach. This approach was used for the City of San Diego's North City Water Reclamation Plant (NCWRP) Pathogen Study (Trussell Technologies 2017a) and evaluated by the City's Pure Water Independent Expert Advisory Panel (IAP) subcommittee (Tchobanoglous and Hardy 2017, NWRI 2019). The Monte Carlo simulation approach involves a statistical analysis of log-normal distributions from both the raw wastewater influent and secondary effluent microbial concentration data to generate a probability distribution model of log removal values for a given microbial target. Since the Monte Carlo simulation estimates the statistical parameters from two independent log-normal variables, any result from the Monte Carlo simulation represents an approximation of the analytical solution defined by the Normality of the LRV calculation (Schwartzman 2018). This approximation was used as one of two statistical strategies to evaluate the most appropriate

interpretation of the acquired microbial concentration data (the other being covariance-based approach described previously).

For the Monte Carlo approach applied to the present study, LRVs for each microbial target were calculated using the MATLAB® software by © 1984-2020 MathWorks, Inc., version R2020a 9.8.0.1359463, equipped with the Statistics and Machine Learning Toolbox. Microbial concentration data were tabulated into a simplified spreadsheet file to facilitate importing values into MATLAB software. Once imported, a statistical model for each influent and effluent dataset for a given microbial target was generated using the maximum likelihood estimates function. These results were then used to run a Monte Carlo simulation for calculating LRVs. In other words, recorded influent and effluent microbial concentrations were used to generate a distribution and from these modeled influent and effluent distributions, one independent and random value was selected from each distribution and subsequently paired to calculate an LRV as shown in Equation 4-4:

$$\mathrm{LRV} = -\log_{10}\left(\frac{C_{eff}}{C_{raw}}\right) \qquad \text{(Equation 4-4)}$$

where: C_{eff} = the concentration of the microbial target taken from the secondary effluent distribution.

C_{raw} = the concentration of the microbial target taken from the raw wastewater distribution.

This calculation was performed 10,000 times to generate a distribution of n = 10,000 LRVs. All LRVs were then sorted from low to high and assigned a rank, *i*, over the total number of data points, *n*. A cumulative probability, *p*, for each value was assigned as shown in Equation 4-5.

$$p = \frac{i-0.375}{n+0.25} \qquad \text{(Equation 4-5)}$$

where: *p* = cumulative probability

i = rank assignment

n = total number of calculated data points

All sorted data were then used to create probability plots for each microbial target. The 5th percentile LRV (or other percentiles) may be read from this plot. The Microsoft Excel percentile formula was used to determine the percentile LRV from the distribution of 10,000+ LRVs. Prior to applying the formula, log removal values were converted to percent removal which is necessary for accurate percentile calculation (Schmidt et al. 2020).

As described further in the study dataset discussion in the next chapter, the Monte Carlo simulation approach used previously for the City of San Diego study was modified for the present study to generate only non-negative LRVs (n = 10,000+ non-negative log removal values). For the present study's dataset for two of the four treatment process, the Monte Carlo

simulation approach generated a fairly large number of negative LRVs as a portion of the total 10,000+ LRVs, which reduced the resulting 5th percentile LRV. It is physically impossible to generate a negative LRV for an enteric virus during wastewater treatment, as virus cannot be created within primary or secondary treatment processes due to the lack of a host organism. Furthermore, the overall removal of virus was evident from the dataset (i.e., influent distribution greater than effluent distribution per probability distribution, see next chapter).

To address the negative LRVs, approximately 1,000 additional LRVs were calculated for a total of 11,000 samples. Negative LRVs within the n=11,000 dataset were removed such that the remaining number of positive LRVs were at least n=10,000. The calculated negative LRVs were believed to be a mathematical artifact of the Monte Carlo's random pairing of influent-effluent values and the characteristics of the current study's underlying dataset. This modified (censored) Monte Carlo approach was used to determine process-specific LRVs, imposing a condition of reality on the statistically determined outcome.

Thus, it may be concluded that for wastewater facility datasets with quite wide-ranging influent and effluent concentration distributions (as shown in the next chapter for the TF and TF/SC processes), the Monte Carlo approach may be inappropriate (or if used requires statistical improvement or modification). This favors the use of other statistical approaches and highlights the need for future work.

CHAPTER 5

Plant Performance and Microbial Monitoring Results

In this chapter, plant operational data, microbial concentration data and calculated LRVs are reported for each secondary treatment process at OC San Plant No. 1 and Plant No. 2. As described in Section 3.5, plant operational performance was monitored during the microbial monitoring period with the assistance of OC San personnel to characterize and describe the normal operating conditions for each secondary treatment process. A statistical review was then performed on specific operational parameters to define a normal operating envelope upon which any awarded LRV GWRS virus credit would be contingent.

To determine log removal, microbial concentration measurements from raw wastewater and secondary effluent samples. were evaluated using both the covariance method and Monte Carlo simulation method to generate probability distributions of log removal values. In this section, LRV results from both statistical approaches are compared and reported.

The results are presented in this section as follows:

- Operating Range Values for each OC San secondary treatment process;
- Microbial concentration data across sites over time;
- Distribution of virus LRVs measured by both the cultivable virus assay and the molecular detection assay for each secondary treatment process as determined by the covariance method and the modified Monte Carlo method;
- Evaluation of the ratio of (relationship between) virus concentrations measured by cultivable virus assay and molecular detection assay across different samples, and comparison of the LRV distributions calculated from the cultivable and molecular datasets;
- Summary of secondary treatment LRVs for influent to the GWRSFE.

All data presented below represent work completed for 24 sampling events, including data obtained from make-up sampling events that occurred between May 2019 through April 2021.

5.1 OC San Operating Range Values for Contingent LRV Credit

Operational parameters and associated values to describe the OC San normal operating envelope were developed and are referred to as operating range values (ORVs). ORVs represent an operating range that defines typical and normal treatment process operations observed during this study. This is applicable to each secondary treatment process supplying influent to OCWD AWPF, i.e., P1 TF, AS1, AS2 (current) and P2 TF/SC (future). An exceedance of a designated ORV would therefore represent a deviation from the normal treatment performance. This deviation could be related to an unexpected event but also due to planned activities such as operational maintenance of treatment systems or flow adjustments by the OC San operators. These ORVs are proposed to be used for contingent LRV credits for GWRS, as with prior pathogen crediting schemes from wastewater treatment for potable reuse in California (Trussell Technologies 2017a). It should be noted that the ORVs are not directly

related to any benchmark, performance goal, or effluent limitation stated in the OC San National Pollutant Discharge Elimination System (NPDES) permit. Failure to meet the ORVs does not signify secondary effluent is of poor quality or unsuitable for reclamation, merely that the effluent does not meet the normal conditions observed during the microbial sampling study and therefore the secondary treatment virus LRV credit is not applicable.

To determine a recommended ORV for each OC San treatment process supplying the GWRS, performance data from P1 and P2 were collected as part of routine plant monitoring during the LRV study sampling period. Performance data were reviewed and analyzed using statistical methods. Based on this analysis, and with assistance from OC San process engineering staff, the following key operational and water quality parameters were selected for each treatment process for developing proposed ORVs, shown in Table 5-1. Additionally, OCWD proposes to utilize ORVs for the GWRS AWPF Microfiltration Feed (MFF) and Microfiltration Effluent (MFE) monitoring locations representing normal GWRS microfiltration influent and effluent quality. Using the described ORVs framework for the overall potable reuse project, each of the four OC San effluents serving GWRS features an ORV, as does the combined (blended) effluents in the form of MFF and MFE, all of which must meet their respective ORVs in order to receive virus LRV credit.

ORVs for each parameter listed in Table 5-1 were calculated using a baseline threshold equation from 30-day average data as follows:

$$\textbf{Upper Baseline Threshold (ORV)} = \textbf{Q3} + \textbf{1.5(IQR)} \qquad \text{(Equation 5-1)}$$

$$\textbf{Lower Baseline Threshold (ORV)} = \textbf{Q1} - \textbf{1.5(IQR)} \qquad \text{(Equation 5-2)}$$

where: Q1 = 25th percentile of the 30-day running average dataset

Q3 = 75th percentile of the 30-day running average dataset

IQR = Interquartile range, defined as Q3 – Q1

The baseline threshold approach was used to define excursions of baseline conditions of a treatment process using a statistical model derived from a large dataset (Debroux et al. 2021). Lower thresholds are defined by values below the 25th percentile by 1.5 times the IQR, while upper thresholds are above the 75th percentile by 1.5 times the IQR, respectively.

Table 5-1. Recommended OC San P1 and P2 Operating Range Values (ORVs) and Plant Performance during Sampling Period.

Treatment Facility	Process	Operating Range Value (ORV)	Plant 30-Day Average Performance During Enteric Virus LRV Sampling Period (5/1/2019 to 4/30/2021)					ORV Breached During Sampling Period?
			Min.	5th	Med.	95th	Max.	
OC San Plant No. 1	AS1 Effluent	**MCRT[(a)] > 3 d** (30-d running ave. of daily values)	4.5	4.6	6.0	7.0	7.1	No
OC San Plant No. 1	AS2 Effluent	**MCRT[(b)] > 4 d** (30-d running ave. of daily values(b))	4.3	4.6	5.5	6.3	6.5	No
OC San Plant No. 1	TF Effluent	**BOD-T < 26 mg/L** (30-d running ave. of daily composite values)	15	16	19	22	24	No
OC San Plant No. 2	TF/SC Effluent	**SRT[(c)] > 1 d** (30-d running ave. of daily values)	1.26	1.3	1.6	1.8	1.9	No
OCWD AWPF	MF Feed	**Turbidity < 10 NTU** (daily average)	OC San specification influent requirement for blended secondary effluent provided to AWPF					No
	MF Effluent	**Turbidity < 0.5 NTU** (instantaneous) **Turbidity < 0.2 NTU** (short term)	Existing limit for MF process pathogen credits (Giardia and Cryptosporidium)					No

Notes: AS = activated sludge, TF = Trickling Filter, SC = Solids Contactor, MCRT = mean cell residence time, BOD-T = total biological oxygen demand, SRT = solids retention time. MCRT (for P1 AS1 and AS2) and SRT (for P2 TF/SC) are calculated daily by OC San using the below equations. OCWD proposes to use the OC San-calculated values of MCRT and SRT for assessment of ORVs related to LRV credit value. BOD-T (P1 TF) is measured by OC San as a daily composite.

(a) MCRT = (Volume of reactor x MLSS) ÷ [(WAS flow x WAS MLSS) + (Effluent Flow x Effluent TSS)]; where MLSS = mixed liquor suspended solids, WAS = waste activated sludge, and TSS = total suspended solids.

(b) Single MCRT values of 0.0 and 109 were recorded during study period for AS2; these values are a result of process maintenance on 9/21/2019 and 9/25/2019, respectively. These extreme values were omitted from the calculation of statistical minimum, median, maximum, and percentile values shown above in order to present the MCRT range during normal operations outside of planned maintenance activity, as well as omitted from the calculation of ORV.

(c) SRT = [(solids contact reactor volume + mixed liquor channel volume) x MLTSS(0.85) + sludge reaeration reactor volume x RTSS(0.85)] ÷ [(WAS flow x WAS MLTSS(0.85) + effluent flow x effluent VSS]; where RTSS = return total suspended solids, WAS = waste activated sludge, MLTSS = mixed liquor total suspended solids; and VSS = volatile suspended solids. No SRT data was reported for P2 TF/SC during the period of 9/1/2019 – 10/31/2019 due to TF/SC basins being out of service for basin maintenance.

The upper baseline threshold for BOD-T is proposed as the ORV for the OC San P1 TF process. This is because higher-than-normal BOD-T in this sampling location would suggest deviations from the typical performance that was documented during the enteric virus sampling. Similarly, the lower baseline threshold for MCRT and SRT is proposed as the ORV for the processes featuring retention time which are the OC San P1 AS1 (MCRT), P1 AS2 (MCRT) and P2 TF (SRT). This is because lower-than-normal MCRT or SRT in these processes would suggest deviation from typical performance.

5.2 Concentration of Microbial Targets at OC San P1 and P2

A plot summarizing enteric virus concentration data over the 24 sampling events acquired by cultivable virus methods (MPN/L) for both raw wastewater and secondary effluent samples is shown below in Figure 5-1. The data is also tabulated in Table 5-2. The data represents all sampling sites from OC San P1 and P2 and includes a subset of events with duplicate sampling datapoints. Three sites feature greater than 24 sampling date data points including P1 raw wastewater (n = 26), P2 raw wastewater (n = 25) and P2 TF/SC effluent (n = 27) because additional native samples were collected to obtain additional matrix spike recovery samples for the April 2021 sampling events.

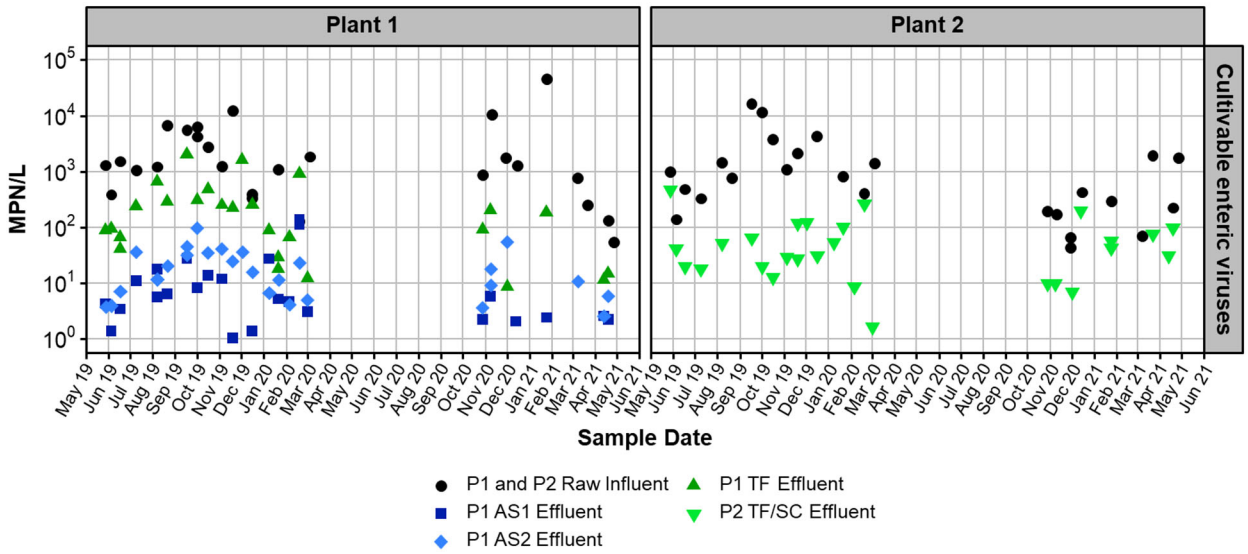


Figure 5-1. Concentrations of Cultivable Enteric Viruses for Raw and Secondary Wastewater Using a Modified Culture Infectivity Assay (EPA 1615).

Data shown above are corrected for virus recovery and include data from OC San Plant No. 1 (left panel) and OC San Plant No. 2 (right panel). The gap in sampling is the SARS-CoV-2 pandemic study interruption.

The cultivable enteric virus concentration range was greater for raw wastewater influent from OC San P1 compared to P2 (Table 5-2). The geometric mean concentration was also greater for OC San P1 than P2. With respect to secondary effluent concentrations, all secondary effluent results for OC San P1 and P2 show measurable enteric virus above the detection limit and at concentrations typically lower than concentrations seen in raw influent samples. These results demonstrate removal of enteric virus at these facilities, though removal varied by treatment process. Removal as LRV is presented in the next section. The geometric mean concentration of cultivable enteric viruses for OC San P1 TF effluent was greater than the other effluents at

$1.3x10^2$ MPN/L, compared to 4.9, $1.3x10^1$, and $3.7x10^1$ MPN/L for P1 AS1, P1 AS2, and P2 TF/SC effluents, respectively (Table 5-2).

Table 5-2. Microbial Target Concentrations Observed in OC San Virus LRV Study.

Microbial Target		Raw Influent		Secondary Effluent			
		P1	P2	P1 TF	P1 AS1	P1 AS2	P2 TF/ SC
Enteric viruses (cultivable), MPN/L	Range	$5.5x10^1$ - $4.5x10^4$	$5.4x10^1$ - $1.6x10^4$	$8.6x10^0$ - $2.0x10^3$	$5.2x10^{-1}$ - $1.2x10^2$	$2.6x10^0$ - $9.7x10^1$	$1.7x10^0$ - $4.8x10^2$
	Median	$1.3x10^3$	$7.9x10^2$	$1.9x10^2$	$4.5x10^0$	$1.2x10^1$	$4.1x10^1$
	Geo.Mean	$1.3x10^3$	$7.5x10^2$	$1.3x10^2$	$4.9x10^0$	$1.3x10^1$	$3.7x10^1$
Enterovirus (molecular assay), GC/L	Range	$1.8x10^4$ - $1.8x10^6$	$1.0x10^4$ - $2.7x10^6$	$1.9x10^2$ - $2.7x10^4$	$7.8x10^2$ - $6.8x10^3$	$7.7x10^2$ - $4.3x10^3$	$7.0x10^2$ - $1.2x10^4$
	Median	$3.0x10^5$	$1.8x10^5$	$1.1x10^4$	$1.7x10^3$	$1.2x10^3$	$2.6x10^3$
	Geo.Mean	$2.5x10^5$	$1.5x10^5$	$6.3x10^3$	$1.8x10^3$	$1.5x10^3$	$2.4x10^3$
Norovirus GII (molecular assay), GC/L	Range	$4.7x10^4$ - $3.8x10^6$	$3.4x10^4$ - $3.8x10^6$	$1.3x10^4$ - $3.1x10^5$	$9.2x10^2$ - $3.7x10^4$	$8.6x10^2$ - $1.1x10^5$	$9.5x10^2$ - $1.1x10^5$
	Median	$6.0x10^5$	$2.7x10^5$	$6.5x10^4$	$4.6x10^3$	$1.4x10^4$	$2.5x10^4$
	Geo.Mean	$5.5x10^5$	$3.0x10^5$	$6.4x10^4$	$5.3x10^3$	$1.2x10^4$	$2.1x10^4$
MS coliphage, PFU/L	Range	$3.6x10^5$ - $3.6x10^7$	$6.6x10^5$ - $1.1x10^7$	$2.9x10^4$ - $2.0x10^5$	$3.9x10^2$ - $2.8x10^4$	$1.2x10^3$ - $1.6x10^4$	$4.9x10^3$ - $1.5x10^5$
	Median	$9.1x10^6$	$2.0x10^6$	$1.2x10^5$	$5.4x10^3$	$4.7x10^3$	$3.8x10^4$
	Geo.Mean	$7.6x10^6$	$2.1x10^6$	$1.1x10^5$	$3.8x10^3$	$4.1x10^3$	$3.1x10^4$
SOM coliphage, PFU/L	Range	$1.9x10^6$ - $1.6x10^7$	$5.3x10^6$ - $2.1x10^7$	$1.2x10^1$ - $5.3x10^6$	$5.4x10^4$ - $1.9x10^5$	$2.1x10^4$ - $2.2x10^5$	$2.6x10^5$ - $2.5x10^6$
	Median	$1.2x10^7$	$1.1x10^7$	$3.4x10^6$	$1.0x10^5$	$1.2x10^5$	$5.6x10^5$
	Geo.Mean	$1.0x10^7$	$1.1x10^7$	$3.0x10^6$	$1.0x10^5$	$9.3x10^4$	$5.7x10^5$
Total coliform, colonies/L	Range	$6.3x10^8$ - $1.4x10^9$	$4.6x10^8$ - $1.4x10^9$	$2.6x10^7$ - $1.3x10^8$	$3.5x10^5$ - $1.8x10^6$	$1.5x10^5$ - $2.4x10^6$	$9.0x10^6$ - $4.6x10^7$
	Median	$8.6x10^8$	$8.8x10^8$	$5.5x10^7$	$7.4x10^5$	$9.3x10^5$	$1.8x10^7$
	Geo.Mean	$8.6x10^8$	$8.6x10^8$	$5.5x10^7$	$7.4x10^5$	$9.0x10^5$	$1.9x10^7$
Fecal coliform, colonies/L	Range	$4.4x10^7$ - $4.4x10^8$	$7.3x10^7$ - $2.7x10^8$	$4.8x10^6$ - $3.5x10^7$	$3.9x10^4$ - $5.5x10^5$	$1.2x10^5$ - $6.1x10^5$	$2.0x10^6$ - $1.4x10^7$
	Median	$2.3x10^8$	$1.8x10^8$	$1.3x10^7$	$2.1x10^5$	$2.8x10^5$	$5.8x10^6$
	Geo.Mean	$2.1x10^8$	$1.6x10^8$	$1.4x10^7$	$1.9x10^5$	$2.7x10^5$	$5.3x10^6$

Uncorrected concentration data obtained using molecular methods to detect enterovirus and norovirus GII are plotted below (Figure 5-2) in gene copies per liter (GC/L) and shown in Table 5-2. Raw wastewater influent concentration ranges of enterovirus were similar between OC San P1 and P2 (Table 5-2). Norovirus GII ranges were also similar between the plants' raw influent. For both plants, the range and geometric means for norovirus GII were greater than for enterovirus in raw influent.

Samples taken from the four secondary treatment sites demonstrate virus genetic material removal at both OC San P1 and P2 (Figure 5-2). For both enterovirus and norovirus GII, geometric mean concentrations were greater in P1 TF effluent compared to P2 TF/SC effluent, followed by the P1 AS effluents (Table 5-2).

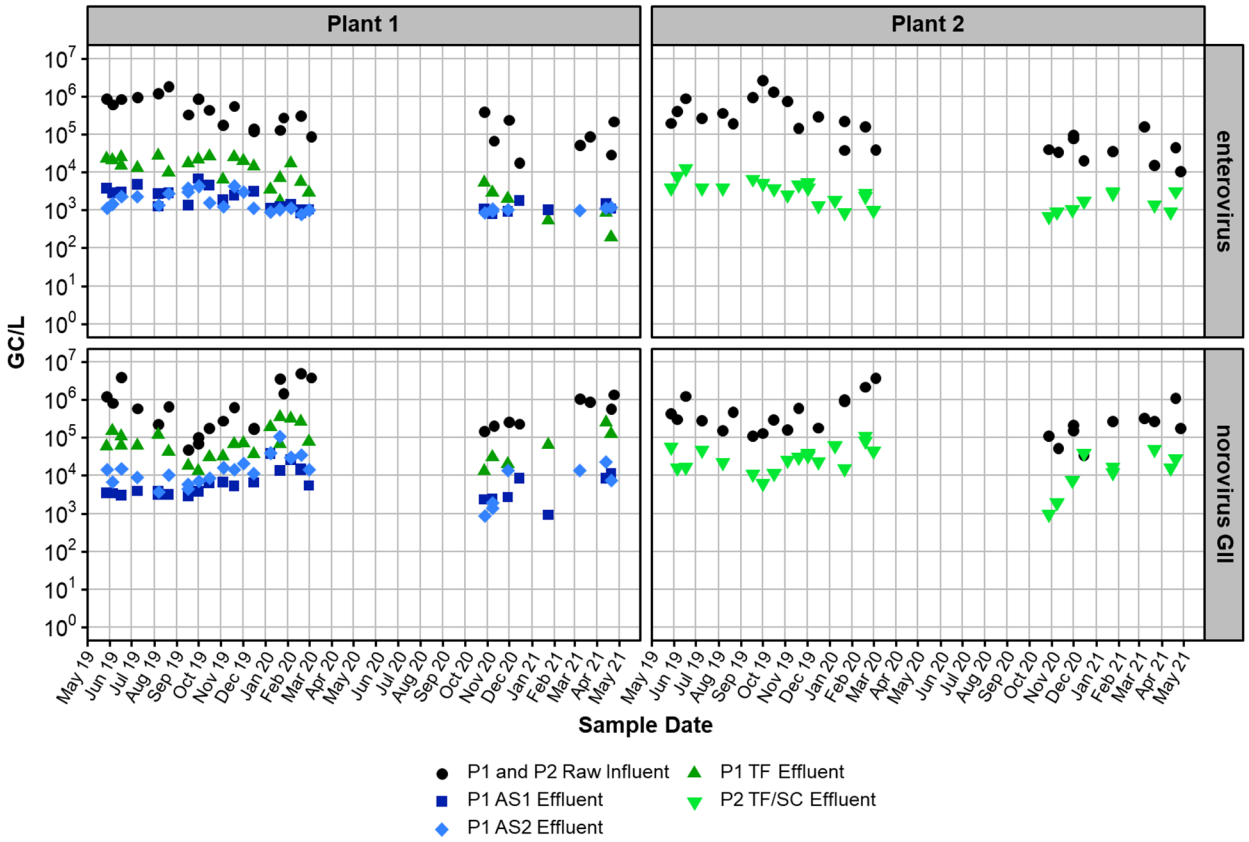


Figure 5-2. Concentrations of Enterovirus and Norovirus GII (ddPCR).
Gene copy detections of enterovirus (top row) and norovirus GII (bottom row) using droplet digital Polymerase Chain-Reaction (ddPCR) for raw and secondary wastewater from OC San Plant No. 1 (left panels) and OC San Plant No. 2 (right panels). The gap in sampling is the SARS-CoV-2 pandemic study interruption.

Data obtained for coliphage targets are plotted below (Figure 5-3) as PFU per liter (PFU/L). MS and SOM coliphage concentration ranges for raw wastewater influent were each similar between OC San P1 and P2, with a wider range observed for MS coliphage compared to SOM coliphage (Table 5-2).

While coliphage concentrations for secondary effluent varied by process, all processes show removal of both MS and SOM coliphage. The AS1 and AS2 processes at OC San P1 show the lowest coliphage geometric mean concentrations (highest removal), while P1 TF effluent demonstrated the greatest geometric mean concentrations.

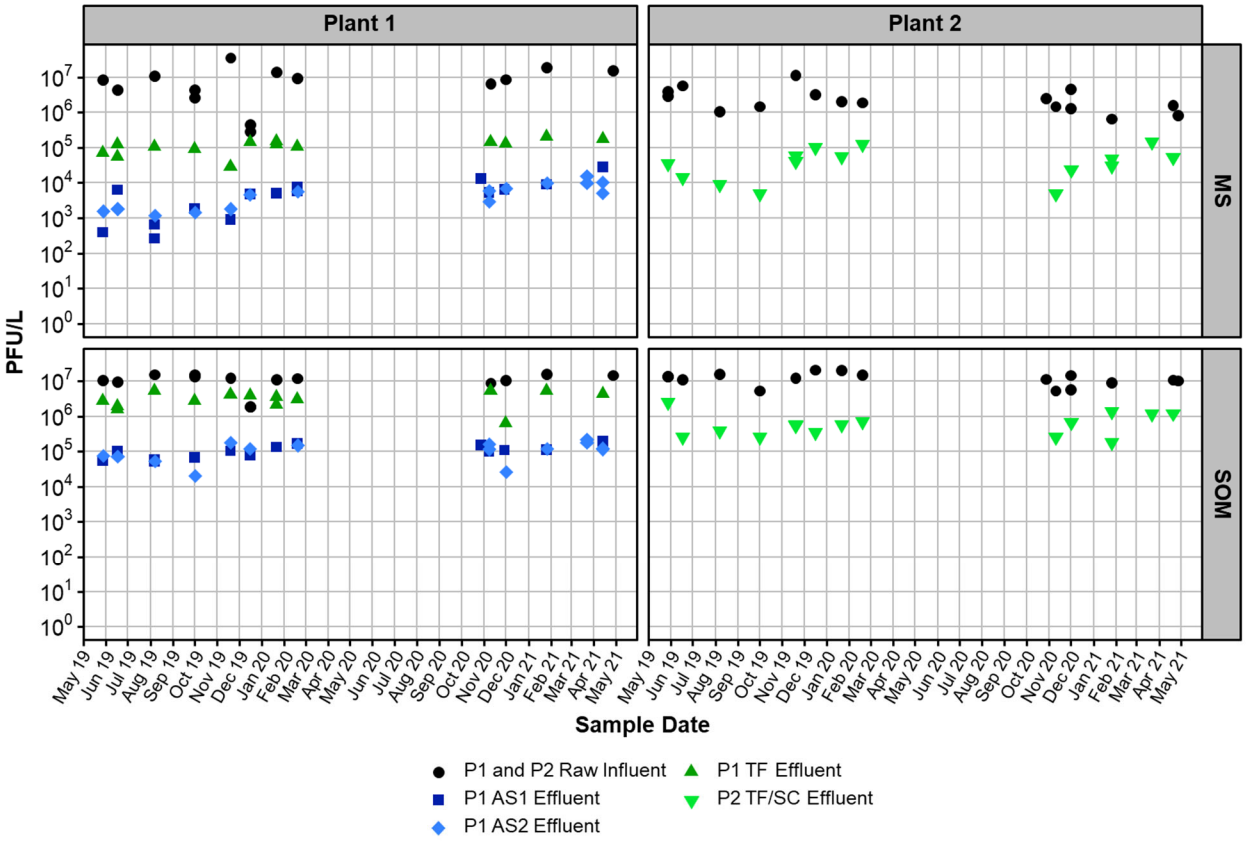


Figure 5-3. Concentrations of Male-Specific (MS) and Somatic (SOM) Coliphage.
Coliphage Data for raw and secondary wastewater from OC San Plant No. 1 (left panel) and OC San Plant No. 2 (right panel) were obtained using a culture-infectivity assay (EPA 1602; double agar overlay). The gap in sampling is the SARS-CoV-2 pandemic study interruption.

Data obtained for fecal and total coliform are plotted below (Figure 5-4) as colonies per liter. Fecal coliform concentration ranges for raw influent were similar between OC San P1 and P2, as were the total coliform concentration ranges. Total coliform geometric mean concentration was greater than fecal coliform for both plants' raw influent.

After secondary treatment at OC San P1 and P2, total and fecal coliform demonstrated a reduction in bacterial concentrations. As observed for all of the other microbial targets in this study, P1 TF effluent exhibited the greatest geometric mean concentration of total and fecal coliform, followed by P2 TF/SC effluent, whereas the AS process effluents exhibited lower concentrations.

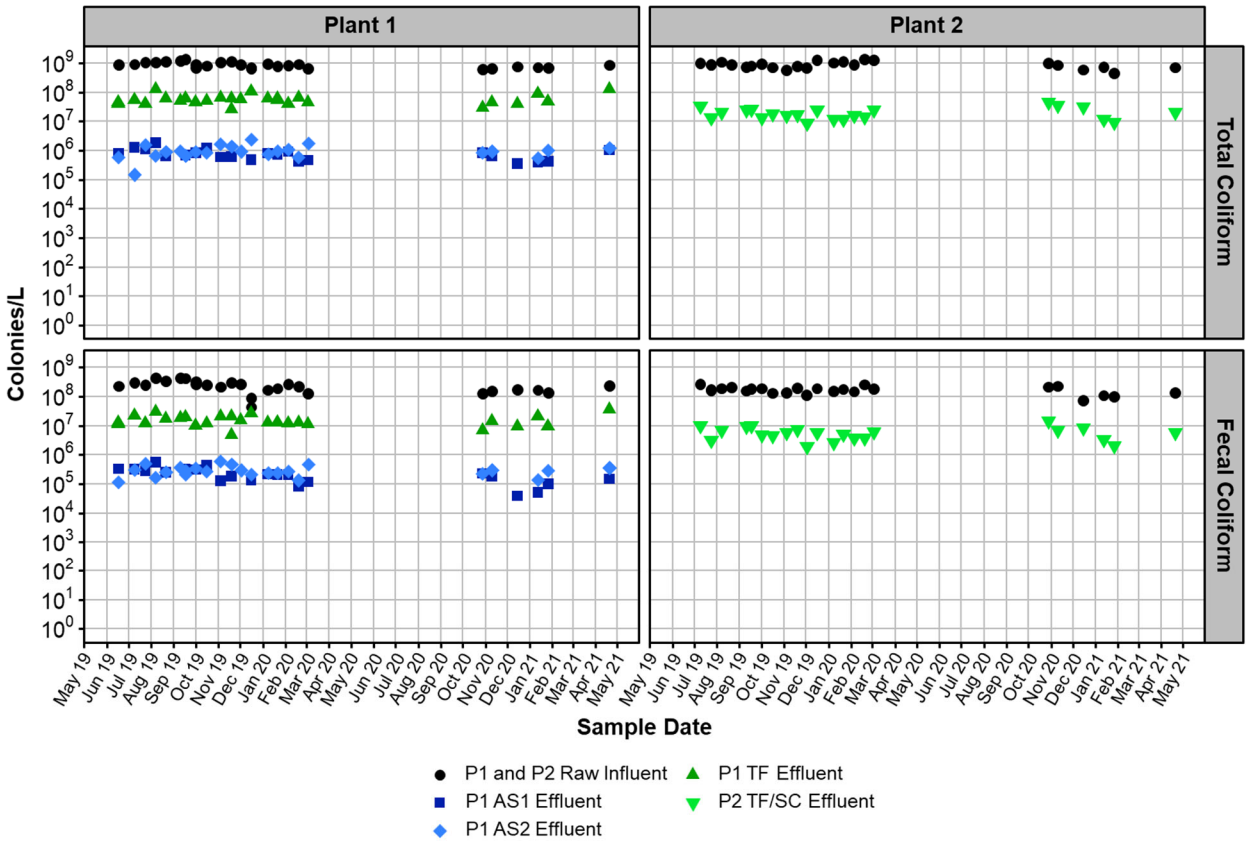


Figure 5-4. Concentration of Fecal and Total Coliform.

Enumeration of fecal and total coliform for raw and secondary wastewater from OC San Plant No. 1 (left panel) and OC San Plant No. 2 (right panel). Sample grabs were analyzed by the OC San laboratory for presence of total coliform (top row) and fecal coliform (bottom row) using SM 9222D and SM 9222B, respectively. The gap in sampling is the SARS-CoV-2 pandemic study interruption.

5.3 Cultivable Enteric Virus Log Reduction

Raw influent and secondary effluent probability plots for cultivable enteric virus concentration data obtained from OC San P1 and P2 are shown below in Figure 5-5. As mentioned previously, no non-detect measurements were observed for any raw influent or secondary effluent sites from P1 and P2. For each sampling site, the distribution of concentration values was fitted with an exponential best-fit line. Note that concentration values are plotted on a cumulative probability plot with a log-scale y-axis to display the best-fit model (see Equation 4-2, Section 4.1). All distributions are best modeled with an exponential best-fit line suggesting that that all data are lognormally distributed.

Using the distribution shown in Figure 5-5, LRVs for each treatment process were calculated using both the covariance and modified Monte Carlo simulation method (see Chapter 4). To plot the LRV distribution as calculated by the covariance analysis method, the 5th percentile, median and 95th percentile values were plotted on a probability plot (Figure 5-6). For the modified Monte Carlo simulation method, a probability plot for the Monte Carlo LRV distribution was plotted using all simulated (n=10,000) LRVs (Figure 5-6). Median and 5th percentile LRVs for both methods are also reported in a summary table below (Table 5-3).

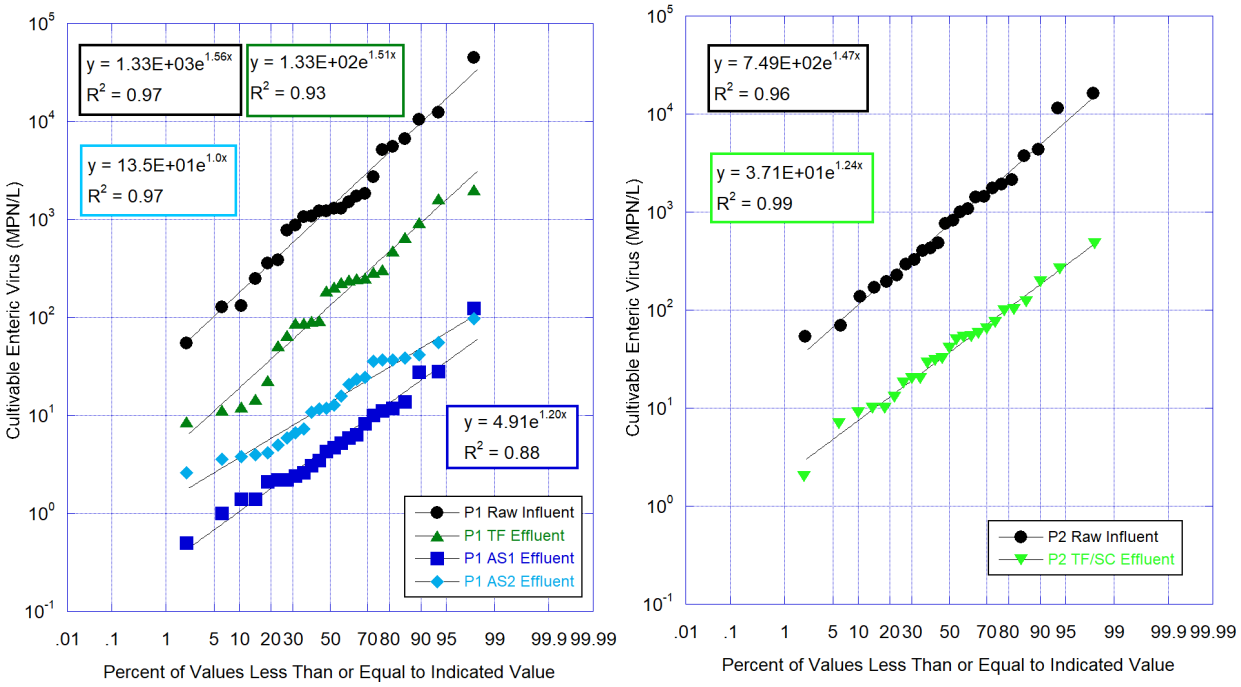


Figure 5-5. Probability Distributions for Cultivable Enteric Virus Concentrations Obtained from Raw Influent and Secondary Effluent Samples Taken at OC San P1 (Left) and P2 (Right).

Each point represents one sampling event and the solid line represents a best-fit regression. The coefficient of determination (R^2 value) is also shown. Raw influent and secondary effluent cultivable virus data obtained from both OC San P1 and P2 are lognormally distributed.

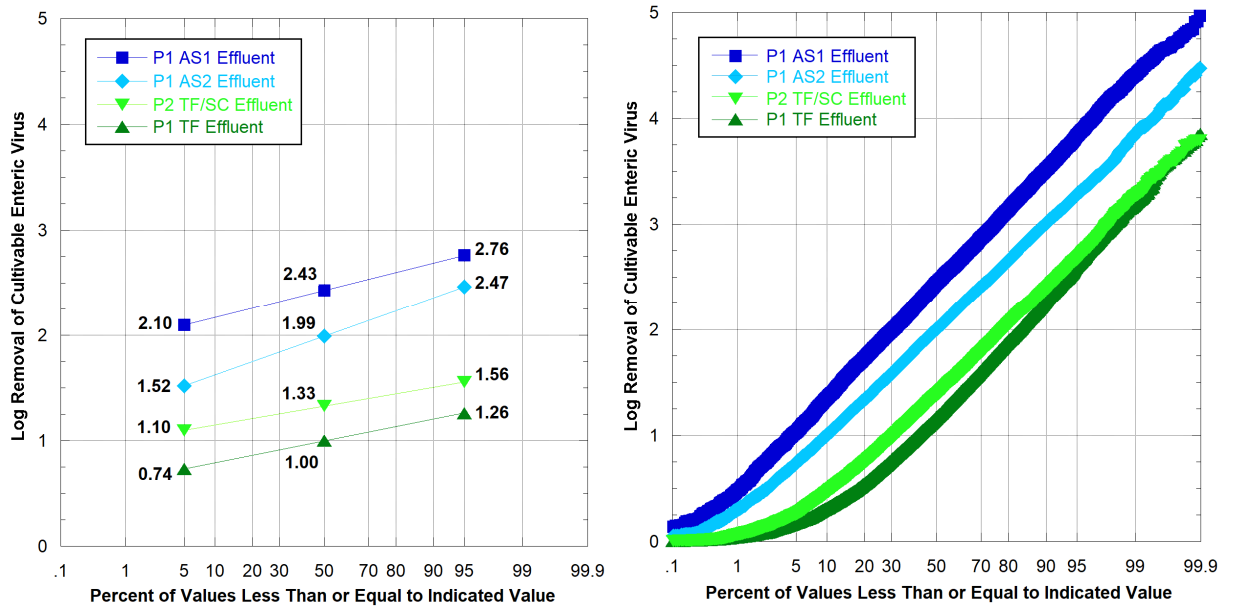


Figure 5-6. Covariance (Left) and Modified Monte Carlo (Right) LRV Distributions Obtained from the Cultivable Enteric Virus Data through Secondary Treatment at OC San P1 and P2.

Table 5-3. Statistical Attributes of the Log Removal Value (LRV) Distributions Generated by Covariance Analysis and Modified Monte Carlo Analysis.

	Microbial Target	Statistic	OC San Plant No. 1			OC San Plant No. 2
			TF	AS1	AS2	TF/SC
Covariance Analysis	SOM coliphage	Median	0.5 (70%)	2.0 (99.0%)	2.1 (99.2%)	1.3 (95%)
		5th percentile	0.4 (61%)	1.7 (98%)	1.8 (98%)	1.1 (92%)
	MS coliphage	Median	1.8 (98.6%)	3.3 (99.96%)	3.3 (99.95%)	1.9 (98.6%)
		5th percentile	1.4 (96%)	2.9 (99.88%)	2.8 (99.86%)	1.5 (97%)
	Enteric Virus (Cultivable Viruses)	Median	1.0 (90%)	2.4 (99.6%)	2.0 (99.0%)	1.3 (95%)
		5th percentile	0.73 (82%)	2.1 (99.2%)	1.5 (97%)	1.1 (92%)
	Enterovirus (ddPCR)	Median	1.6 (97%)	2.1 (99.3%)	2.2 (99.4%)	1.8 (98%)
		5th percentile	1.3 (95%)	1.7 (98%)	1.7 (98%)	1.3 (94%)
	Norovirus GII (ddPCR)	Median	0.9 (88%)	2.0 (99.0%)	1.7 (98%)	1.1 (93%)
		5th percentile	0.7 (78%)	1.7 (98%)	1.7 (98%)	0.8 (86%)
Modified Monte Carlo Analysis	SOM coliphage	Median	0.6 (72%)	2.0 (99.0%)	2.1 (99.1%)	1.2 (94%)
		5th percentile	0.1 (22%)	1.5 (97%)	1.4 (96%)	0.5 (67%)
	MS coliphage	Median	1.8 (98.6%)	3.3 (99.95%)	3.3 (99.94%)	1.8 (98.5%)
		5th percentile	0.98 (90%)	2.1 (99.2%)	2.3 (99.5%)	0.9 (87%)
	Enteric Virus (Cultivable Viruses)	Median	1.2 (93%)	2.4 (99.6%)	2.0 (99.0%)	1.4 (96%)
		5th percentile	0.18 (33%)	1.0 (91%)	0.7 (82%)	0.3 (45%)
	Enterovirus (ddPCR)	Median	1.6 (98%)	2.1 (99.3%)	2.2 (99.4%)	1.8 (98%)
		5th percentile	0.4 (64%)	1.2 (94%)	1.3 (95%)	0.7 (80%)
	Norovirus GII (ddPCR)	Median	1.0 (90%)	2.0 (99.0%)	1.7 (98%)	1.2 (93%)
		5th percentile	0.2 (33%)	0.96 (89%)	0.6 (75%)	0.3 (46%)

Notes: Log removal values (also shown in the form of percent removal in parenthesis) were calculated using recovery-corrected values for SOM coliphage, MS coliphage, and enteric virus (cultivable viruses). LRVs for enterovirus and norovirus GII (ddPCR) were calculated using uncorrected values.

Using the covariance approach, the median LRV for cultivable enteric viruses at OC San P1 TF and P2 TF/SC was 1.0 and 1.3, respectively. The median LRVs for P1 AS1 and P1 AS2 were greater at 2.4 and 2.0, respectively. Using the modified Monte Carlo approach, the median LRV was quite similar to the covariance approach at 1.2 and 1.4 for cultivable enteric viruses at OC San P1 TF and P2 TF/SC, respectively, and 2.4 and 2.0 for P1 AS1 and P1 AS2, respectively (see Figure 5-6 or Table 5-3).

While the median LRVs were found to be similar between the two calculation approaches across the different treatment processes, the 5th percentile LRV is greater for covariance

approach, consistent with the greater steepness of the LRV distribution for the modified Monte Carlo approach (Figure 5-6, right). The 5th percentile values are summarized in Table 5-3. The lower 5th percentile LRVs for modified Monte Carlo is related to the random pairing of relatively high effluent concentration values with unrelated low influent values. Conversely, the higher 5th percentile LRVs observed for the covariance approach is related to how influent and effluent concentration values are correlated (dependent). This correlation is illustrated below in Figure 5-7, which demonstrates how ranked log concentration values from the influent and effluent are related. Correlations for each secondary effluent process were modeled with a linear trendline to calculate Pearson's correlation coefficient of determination (R^2). R^2 values measure the amount of variation between two distributions and range between -1 and 1, where 0 represents no correlation and 1 or -1 represent a perfect correlation. Each correlation shown in Figure 5-7 is statistically significant, with probability values (p-values) less than 0.1% ($p < 0.001$), as shown in Table 5-4.

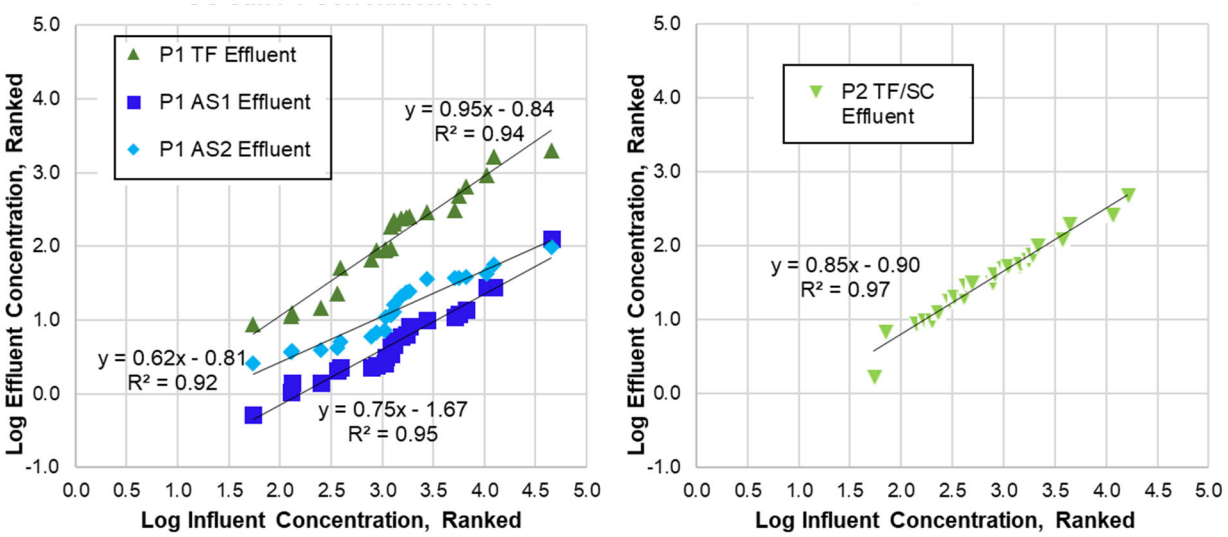


Figure 5-7. Log Influent and Effluent Concentration Correlation Plots for OC San P1 (Left) and P2 (Right). Ranked concentration values (n=24) from each P1 secondary treatment process (P1 TF, AS1, AS2) were compared to ranked raw influent concentration values for P1 (left). Similarly, ranked P2 TF/SC secondary effluent concentration values (n=24) were compared to ranked raw influent concentration values from P2 (right). The linear model represents the correlation between each distribution from which the correlation coefficient of determination (R^2) was calculated.

Table 5-4. Probability Values (p-values) for OC San P1 and P2 Raw Influent and Secondary Effluent Correlations.

OC San Treatment Process	R^2	p-value
P1 TF	0.945	<0.001
P1 AS1	0.952	<0.001
P1 AS2	0.919	<0.001
P2 TF/SC	0.966	<0.001

The GWRS IAP deemed the covariance approach to be preferable over the Monte Carlo approach including considering the Monte Carlo modification. Per comments received by the GWRS IAP, the Monte Carlo approach was considered state-of-the-art at the time that the

present study's test plan was developed. However, since completing the data collection, approaches for calculating conservative LRV estimates for wastewater treatment have evolved.

When reviewing the overall study results for LRVs such as in above Table 5-3, it should be noted that differences in LRVs that may appear significant (e.g., 1.0 versus 0.7) are not large when considering the result in terms of percent removal especially as LRV increases (e.g., 1.0 and 0.7 LRV correspond to 90% and 80%, respectively; or 2.0 and 1.5 LRV correspond to 99% and 97%, respectively). Thus, apparent differences in removal between different microbial targets or treatment processes in this study are sometimes not significant which should be considered during interpretation.

5.4 Molecular Detection of Enteric Viruses and Log Reduction

Uncorrected molecular concentration data acquired from EPA Method 1615 using ddPCR analysis (see Section 3.2) for detection of enterovirus and norovirus GII genetic material were used to generate similar probability distributions as described above, shown below in Figure 5-8. As before, the distribution of concentration values is fit with an exponential best-fit line and show that all data are best modeled as a lognormal distribution.

Non-detect measurements were observed for a subset of sampling events for enterovirus including P1 AS1 (n=8), P1 AS2 (n=11), and P2 TF/SC (n=5) secondary effluent. Only one non-detect measurement was observed for norovirus GII for one sample of P1 AS2 effluent. These cases were substituted with the method detection limit as a conservative upper end estimate of the effluent concentration for purposes of completing the LRVs statistical determination; use of a statistical technique to estimate values below the detection limit would result in comparatively lower effluent concentrations assigned to the non-detect results and correspondingly higher LRVs.

Covariance and modified Monte Carlo simulation LRV distributions generated for each treatment process are presented in Figure 5-9 for enterovirus and norovirus GII. A summary of median and 5th percentile enterovirus and norovirus GII LRVs can also be found above in Section 5.3, Table 5-3. Irrespective of the statistical method used, the least efficient secondary treatment process for both enterovirus and norovirus GII is OC San P1 TF, which is consistent with other targets measured in this study (cultivable virus, SOM coliphage and coliform). Of all the treatment processes, the AS secondary treatment processes at OC San P1 demonstrates the highest removal of gene copies.

Similar to the cultivable virus, 5th percentile covariance LRVs for both enterovirus and norovirus GII are larger than the Monte Carlo 5th percentile LRVs. For covariance approach for enterovirus, the P1 TF and P2 TF/SC show the same 5th percentile LRV (rounding to 1.3 LRV) while the modified Monte Carlo analysis shows P1 TF as the lowest 5th percentile LRV. It should be noted that since the method detection limit was substituted in place of non-detect for several samples in the case of enterovirus analysis, this may affect the correlation variable in the covariance analysis method, which may explain the converging distributions at the lower percentile (5th percentile) LRVs.

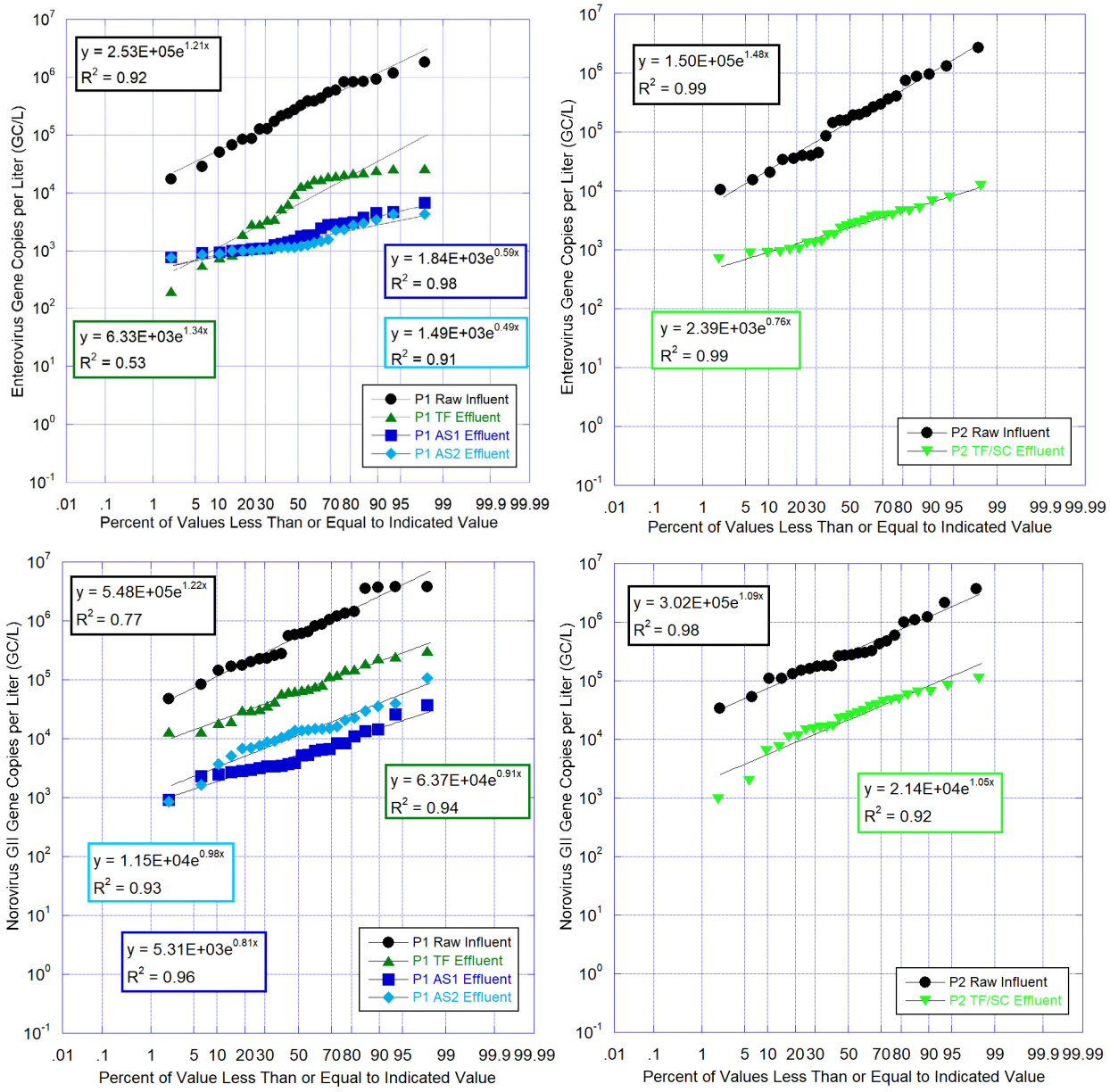


Figure 5-8. Probability Distributions for Enterovirus (Top) and Norovirus GII (Bottom) Concentrations from Droplet Digital PCR (ddPCR) Analysis of Raw Influent and Secondary Effluent Samples Taken at OC San P1 (Left) and P2 (Right).

Each point represents one sampling event the dashed line represents a best-fit regression. The coefficient of determination (R^2 value) is also shown. Raw influent and secondary effluent molecular assay data obtained from both OC San P1 and P2 are lognormally distributed.

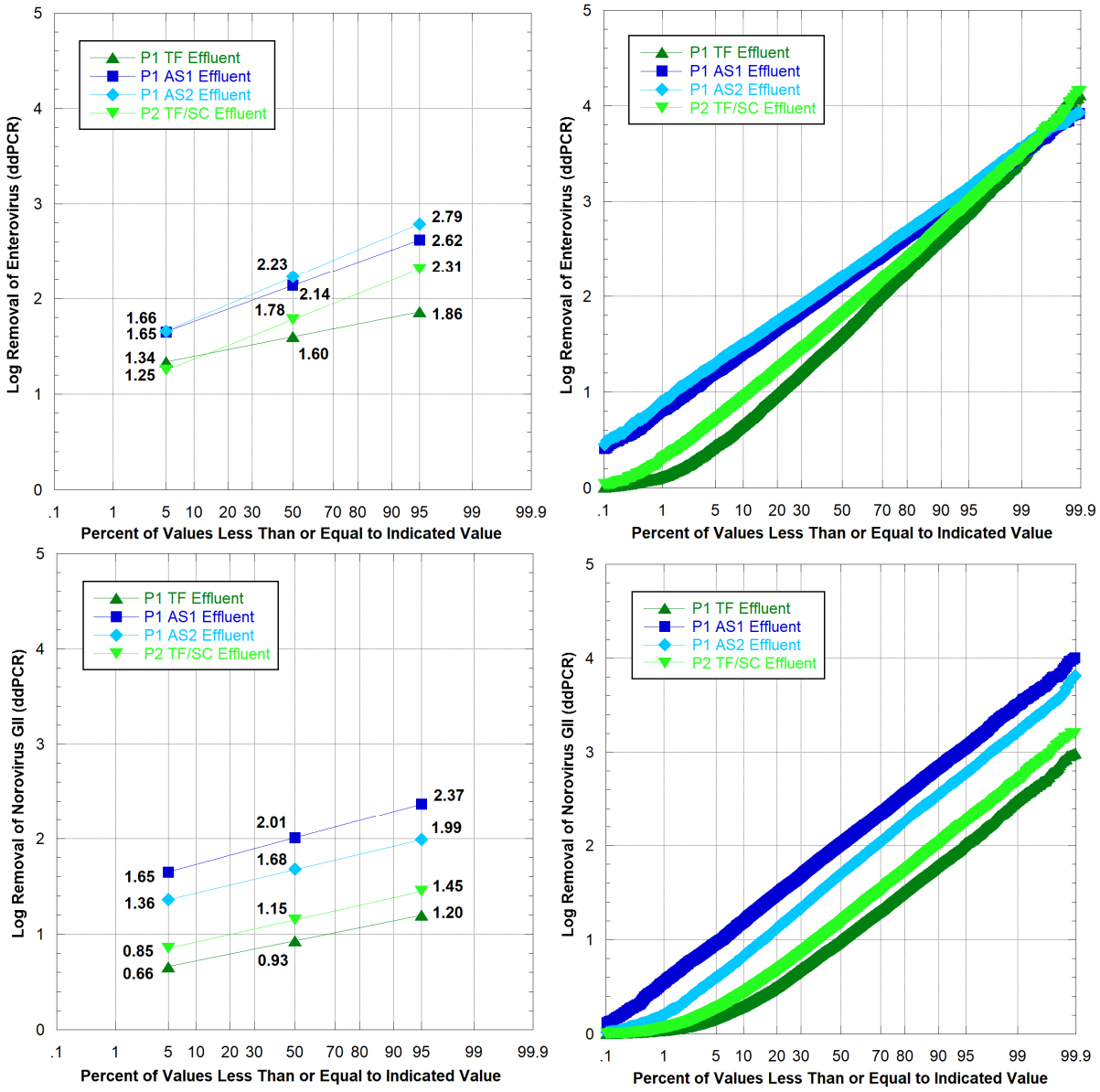


Figure 5-9. LRV Distributions Using Covariance Analysis (Left) and Modified Monte Carlo Simulations (Right) Obtained from Droplet Digital PCR (ddPCR) Assay for Detection of Enterovirus (Top) and Norovirus GII (Bottom) through Secondary Treatment at OC San P1 and P2.

5.5 Comparison of Molecular and Cultivable Enteric Virus Measurements

One objective of this study was to determine how molecular detection of enterovirus and norovirus GII compares to detection of total cultivable enteric viruses measured by the infectivity method. Molecular detection methods entail amplification and quantitation of genetic material present in a sample while the cultivable detection assay relies on infectivity of viable viruses present in the sample (Section 3.1). Each method has specific advantages in that ddPCR can quantitate specific enteric viruses that cannot be propagated via cell-culture vectors, while cultivable methods can directly assess viability (and infectivity) of a subset of enteric viruses.

Results from molecular methods used to assess virus occurrence are not directly tied to virus infectivity, since the molecular assay only measures the occurrence of a section of the genome and therefore will not detect damage that has occurred at other locations (Bartolo 2018, Trussell Technologies 2017a, b). In other words, occurrence of gene copies at a given concentration does not imply that the corresponding viruses are intact or infectious.

Data were compared to determine if any relationship exists between the two assays in the OC San dataset with respect to observed measurements from the cultivable enteric virus assay (enumerated as MPN/L) and the molecular detection method using ddPCR (enumerated as GC/L). To this end, a ratio of the two measures was calculated as performed in other studies (Trussell Technologies 2017a). The ratio of enterovirus (GC/L) and cultivable virus (MPN/L) was calculated for raw influent measurements from samples taken on the same sampling day, for OC San P1 and P2. A similar analysis was done for secondary effluent for each sampling day for OC San P1 TF, AS1, AS2, and P2 TF/SC effluents and plotted below in Figure 5-10.

The plotted ratios of enterovirus data (GC/L) to cultivable virus data (MPN/L) show that there is no consistent relationship between the observed measurements. A strong relationship between each assay should be illustrated by a relatively constant ratio which would appear as a horizontal line in Figure 5-10. This is not observed for any of the wastewaters tested. The ratio varies by up to 3 orders of magnitude for P1 raw wastewater, P2 raw wastewater, P1 TF effluent, and P1 AS1 effluent. While the ratios calculated for P1 AS2 effluent and P2 TF/SC effluent vary by only 2 orders of magnitude, these ratios also fail to demonstrate a consistent trend. In addition, these large ratio values indicate that the values observed for molecular methods (GC/L) are higher than values observed via cultivable methods (MPN/L), with the exception of one instance for P1 TF effluent recorded in February 2020.

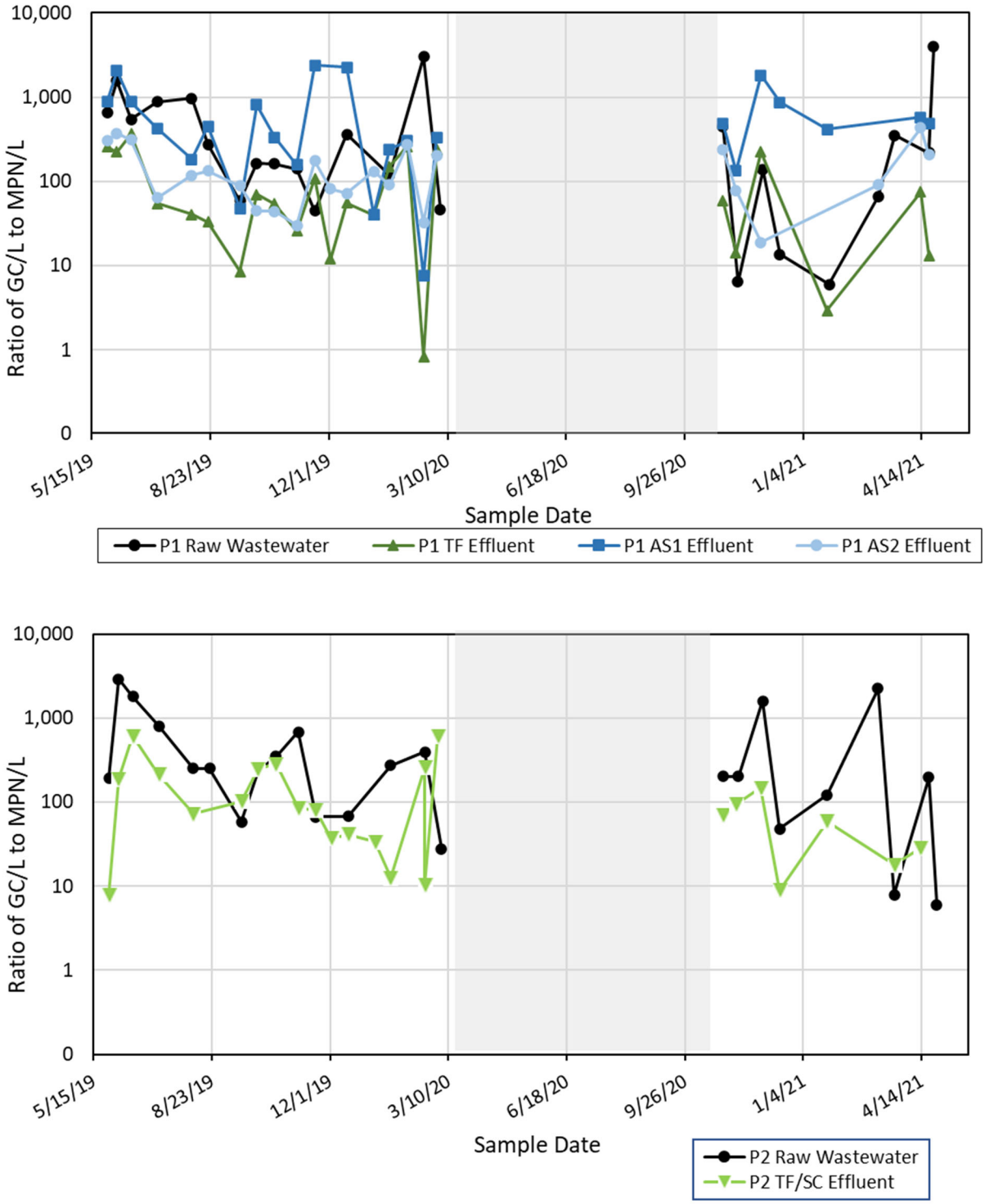


Figure 5-10. Ratio of ddPCR Enterovirus (GC/L) to Cultivable Virus (MPN/L) in OC San P1 (Top) and P2 (Bottom).

Grey block represents the period of time when the MSU laboratory was shut down due to the coronavirus pandemic.

To further compare the two virus assay methods, Figure 5-11 presents the covariance and modified Monte Carlo LRV distributions calculated from the cultivable virus dataset and the

molecular (ddPCR) dataset for enterovirus on a probability distribution plot for each secondary treatment process. These distributions were previously presented in Sections 5.3 and 5.4 but are here plotted side-by-side to highlight similarities and differences between each respective distribution. Note that a comprehensive table listing LRVs for each OC San treatment process can be found earlier in Table 5-3.

Overall, the Monte Carlo distributions exhibit a wide range of LRVs for both cultivable and molecular assays and have lower 5th percentile LRVs than the covariance method for all treatment processes. Compared to the Monte Carlo distributions, covariance distributions for both the cultivable and molecular assays have relatively flatter slopes and therefore a narrower range of LRVs, with greater LRVs at the low percentile ranks. Despite these differences in slope, the median LRVs for a given virus target (cultivable or molecular) are similar regardless of the statistical method used. Cultivable and molecular assay datasets show the highest similarity in distribution of LRVs for P1 TF data, as seen by the parallel lines, for Monte Carlo and covariance methods. This is also true for P2 TF/SC Monte Carlo LRV distributions, but less so for the covariance distribution. Crossover of the Monte Carlo distributions for P1 AS1 and AS2 indicates that the molecular assay only exhibits greater removal below a certain percentile. In contrast, the covariance distributions do not exhibit crossover for any process or microbial target. Overall, the LRV distributions for the covariance method showed greater LRV for molecular assay than cultivable, except for P1 AS1. Thus, in this study, both the Monte Carlo and covariance LRV calculation methods show that the molecular assay for enterovirus typically featured a greater LRV than the cultivable assay for a given percentile, but not always. The cases where the molecular assay demonstrated a lower LRV than the cultivable assay was strictly for the AS treatment processes, and never for the P1 TF or P2 TF/SC processes.

It should be noted that in this study, as described in Section 3.4, the cultivable data were recovery-corrected whereas the molecular assay data were not; thus, definitively comparing LRVs trends between the two targets is uncertain if ddPCR recovery is significantly different between raw influent versus secondary effluent. If recovery is similar, recovery correction is not as necessary since mathematically identical recoveries of influent and effluent would not affect the removal calculation comparing influent and effluent. However, it is likely that recoveries of influent and effluent samples are not identical given the variability that was observed in this study for MS and SOM recovery (Table 3-4, Section 3.4).

Overall, based on the above observations from this study, additional research and optimization is needed if molecular data is to be relied upon by other future studies evaluating virus LRV to improve the quality of the molecular dataset.

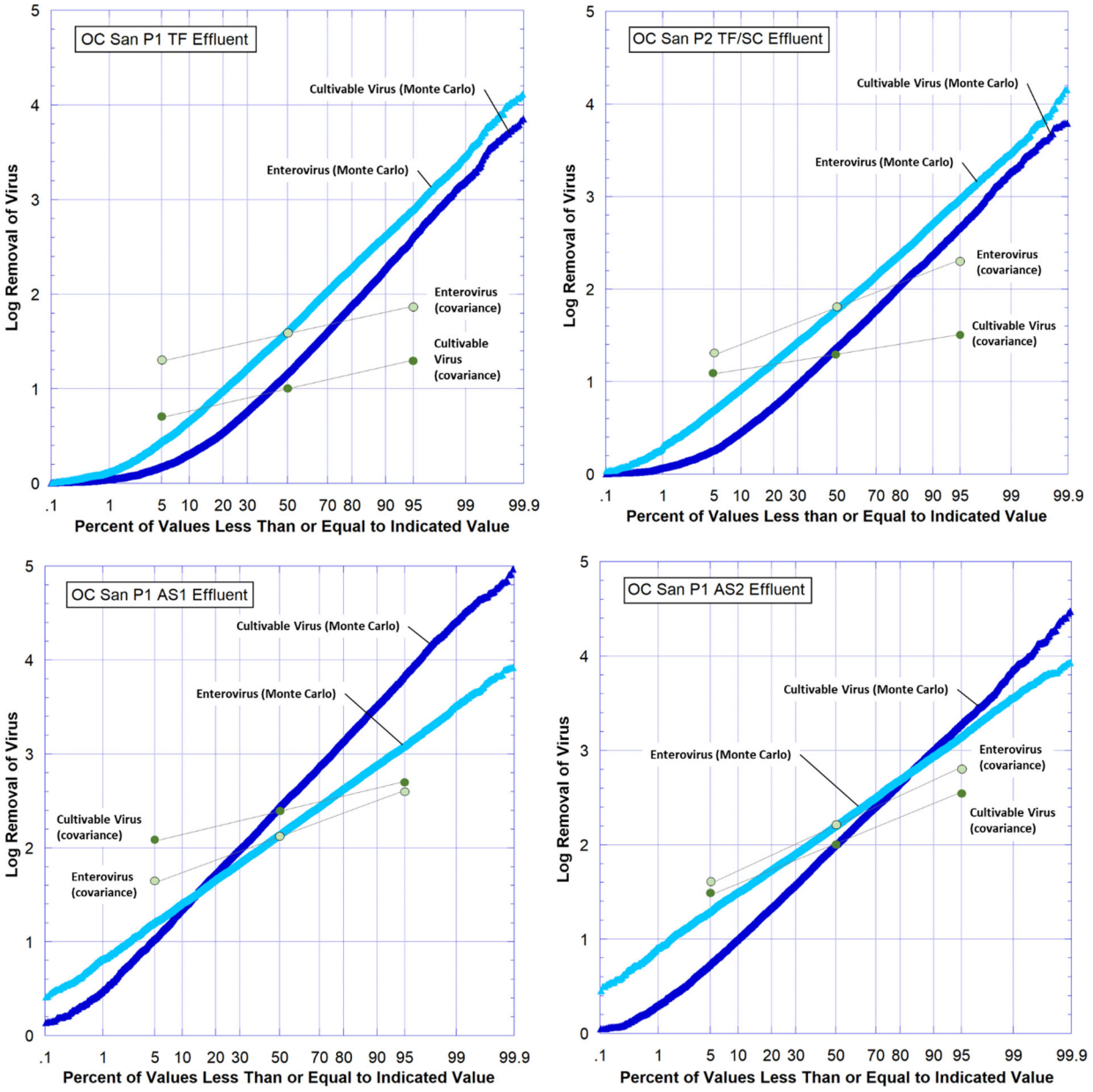


Figure 5-11. Comparison of Covariance and Modified Monte Carlo LRV Distributions Calculated from the Enterovirus Molecular Assay (ddPCR) Dataset and the Cultivable Virus Dataset.

An overlay of all LRV distributions is shown above for (clockwise from upper left) OC San P1 TF, P2 TF/SC, P1 AS2, and P1 AS1 effluents. Covariance LRV distributions for the enterovirus target (light green circles) and cultivable viruses (dark green circles) are shown. Modified Monte Carlo LRV distributions (n>10,000) for the enterovirus target (light blue) and cultivable viruses (dark blue) are also plotted.

5.6 Summary of Microbial LRV Probability Distributions

To compare the degree of removal for each microbial target across each treatment process, a summary of the LRVs associated with each distribution is listed in Table 5-3 and Figure 5-12 below. For Table 5-3, results are shown for both described statistical approaches (covariance method and modified Monte Carlo simulations). Figure 5-12 presents a subset of these same results focused only on the covariance method for each microbial target.

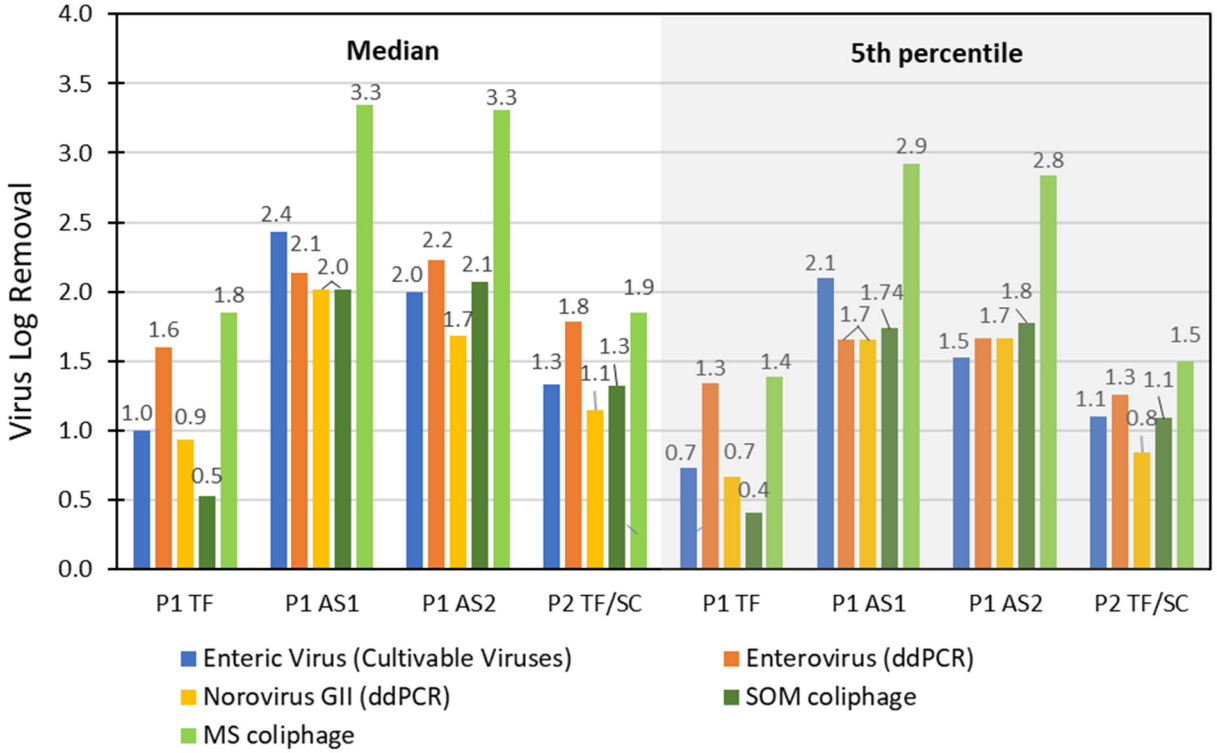


Figure 5-12. Summary of the Log Removal Values (LRVs) Generated by the Covariance Analysis Approach for Cultivable Viruses, Enterovirus (ddPCR) and Norovirus GII (ddPCR), MS Coliphage, and SOM Coliphage.

Key observations regarding the measured LRVs include:

- For LRVs obtained using the covariance approach for cultivable virus and all other microbial targets, the P1 TF and P2 TF/SC processes exhibit lower median and 5th percentile LRVs than P1 AS1 and AS2. In addition, P1 AS1 and AS2 show similar LRVs for a given target. Overall, the P1 TF process typically had the lowest 5th percentile LRV. This result is also true for LRVs calculated using the modified Monte Carlo method, with exception to MS coliphage for P1 TF/SC, which showed marginally higher removal than P2 TF/SC. Overall, results for calculated removal of all microbial targets is consistent with the fact that the P1 TF effluent exhibited higher microbial target concentrations for all targets compared to the other three effluents (Table 5-2) and the general degree of treatment expected from each process.
- For ddPCR target LRVs in Table 5-3, the lowest median LRV was P1 TF and P2 TF/SC, whereas P1 AS1 and AS2 showed higher and similar LRVs, consistent with the median LRVs for cultivable virus, despite the inherent differences in measuring total genetic material (ddPCR) and viable virus (cultivable). For both median and 5th percentile LRVs, norovirus GII LRV was always slightly lower than cultivable virus LRV for all treatment processes except for the 5th percentile LRV for P1 AS2, which was slightly higher. Conversely, both median and 5th percentile LRVs for enterovirus ddPCR were typically slightly higher than the 5th percentile cultivable virus LRV, except for P1 AS1. Other studies have shown greater removal of genetic material over cultivable virus at the 5th percentile and mean LRV (NWRI 2019, Trussell Technologies 2017a). The variability of LRVs above between the two assays could be related to the present study's use of ddPCR instead of qPCR as well as the lack of

recovery correction for the ddPCR results. Further, evaluation of split samples measured by both methods in the present study reveals inconsistent relationship between results from the two assays (see Section 5.5).

- While the median values for LRV are similar between the two statistical analysis approaches used to determine LRVs, at the 5th percentile the modified Monte Carlo analysis approach results in a lower LRV than the covariance analysis approach (i.e., 0.18 versus 0.73, respectively, for cultivable virus P1 TF LRV). This is consistent with fundamental differences between these two statistical methods. A portion of the time, the modified Monte Carlo simulation randomly pairs raw influent and secondary effluent concentration values that are quite similar (based on the overlapping concentration ranges observed for this study for some treatment processes and microbial targets over the 24 sampling events) and these cases produce the lowest LRVs in the distribution thus driving the 5th percentile value. In contrast, the covariance method generates its LRV probability distribution by pairing influent and effluent concentration data by simple rank order of the 24 sampling events to estimate the sample mean. With this approach, which is preferred by the GWRS IAP because it assumes dependence rather than independence between the influent and effluent datasets, the lowest influent concentration value is never paired with the highest effluent value as in the Monte Carlo simulation.

In summary, enteric virus, MS and SOM coliphage, and total and fecal coliform concentrations were determined for OC San's raw wastewater influent at both OC San Reclamation Plant No. 1 (P1) and Treatment Plant No. 2 (P2) in the following four secondary effluents: OC San P1 activated sludge (AS) effluents (AS1 and AS2), P1 trickling filter (TF) effluent, and P2 trickling filter/solids contactor (TF/SC) effluent. Enteric virus log removal values (LRVs) for the P1 and P2 wastewater treatment facilities were then determined using two different statistical techniques for the three secondary effluents currently relied upon as source waters for the OCWD GWRS potable reuse facility as well as for a fourth secondary effluent (from P2) that will be blended with these P1 effluents as an additional source water following completion of the GWRS Final Expansion. The statistical techniques consisted of a covariance approach and a modified Monte Carlo statistical approach each applied to calculate LRV for each process.

The overall purpose was to determine an appropriate and conservative estimate of the enteric virus reduction to credit the wastewater treatment process that precedes advanced treatment and contributes to overall reduction in virus during water reclamation. Based on precedent from past California studies, DDW recognizes the 5th percentile LRV as the preferred statistically conservative pathogen removal value on which to base an awarded credit value, as opposed to the mean or median (50th percentile). For this study, based on the covariance approach, a conservative 5th percentile LRV of 0.73 (82% removal) was proposed to DDW as the virus log removal credit value. This was based on the lowest 5th percentile LRV observed for the four wastewater treatment processes monitored. OCWD has proposed the 0.73 LRV for virus credit for wastewater treatment, along with the contingent ORVs described in Section 5.1 related to OC San operating envelope, as part of the GWRSFE Title 22 Engineering Report. At the time of this report, DDW is reviewing the OCWD proposal.

Some general conclusions and recommendations from this study are described in Chapter 7, for the potential benefit of any other potable reuse agency seeking to evaluate log removal and obtain pathogen credits for wastewater treatment, based on the observations from this study.

CHAPTER 6

Study Comparison to WRF Project 4989 (DPR-2)

As part of a state of California initiative to address knowledge gaps for developing criteria for direct potable reuse (DPR), six research projects were identified and designed to address and develop DPR criteria. The second project (DPR-2) has been completed by Trussell Technologies and focused on assessing the concentrations and variability of microbial pathogens in raw wastewater by evaluating raw wastewater samples collected from five different WWTPs and developing recommendations for sample collection and future monitoring efforts (Pecson et al. 2021). The DPR-2 study is an independent study from the study described herein. Given the significant expertise involved in planning and executing the DPR-2 project and that its ultimate recommendations are intended to benefit projects in the state, for the present study OCWD aimed to remain consistent with DPR-2 project-selected sampling practices for raw wastewater evaluation. Thus, raw wastewater sampling for the present OC San LRV study was designed to reflect strategies employed by DPR-2. Below is a description of similarities and differences between this study and the DPR-2 study.

6.1 Comparison of Sampling Methods and Analytical Targets

DPR-2 measured the concentrations of specific viruses and other pathogens including *Cryptosporidium*, *Giardia*, enteric viruses (including norovirus which is analyzed by molecular detection methods), and MS/somatic coliphage from five different WWTPs (Pecson et al. 2021). As a result of the pandemic, the SARS-CoV-2 virus was added to the virus-target scope of work. One of the five treatment plants that participated in the DPR-2 study was OC San P1. Raw wastewater samples collected for the DPR-2 study were collected independently from raw wastewater samples collected for the OCWD-OC San LRV study, and data from DPR-2 was not combined with the data collected for the OC San LRV study for the LRV determination. The overall DPR-2 scope includes a total of 120 raw wastewater samples (24 samples per WWTP) analyzed by three independent laboratories. One of the participating laboratories was BCS, the same laboratory that performed cultivable virus analysis in the OCWD-OC San LRV study. Significant differences between the OC San LRV study and DPR-2 are (1) DPR-2 focuses exclusively on raw wastewater (i.e., WWTP influent) and does not include sampling of treated effluent; (2) DPR-2 includes enumeration of SARS-CoV-2, *Giardia* cysts and *Cryptosporidium* spores (these are not included in the scope of work for this study); and (3) DPR-2 acquired microbial pathogen data from three independent laboratories whereas the present study only acquired data from a single laboratory (BCS Laboratories).

For both the OC San LRV study and the DPR-2 study, the concentration of enteroviruses and norovirus GII present in raw wastewater were determined using EPA method 1615 for cultivable and molecular detection of enteric viruses. All cultivable and molecular concentration data from both studies were lognormally distributed. With exception of one DPR-2 sample reported as ‘detected but not quantifiable’, the range and distribution of cultivable enteric virus concentration data between both the OC San LRV study and the DPR-2 study (for the OC San site) were very similar. In contrast, the studies showed slight differences in raw concentration

data obtained using molecular detection assays. While the overall range and distribution of enterovirus concentration data from both the OC San LRV study and DPR-2 study were very similar, approximately eight non-detects were reported by DPR-2. Furthermore, four non-detects and 12 detected-but-not-quantifiable results were reported by DPR-2 for norovirus GII molecular concentration data. When comparing the OC San LRV and DPR-2 norovirus GII concentrations, it is apparent that both the range and distribution of concentration values are different. No non-detects were reported for the OC San LRV study for either enterovirus or norovirus GII. It is unclear whether non-detects contributed to differences in the norovirus GII distribution. These differences between non-detect samples may be attributed to the use of a qPCR assay by the DPR-2 study while the OC San LRV study used a ddPCR assay. No comparisons can be made for additional virus targets since virus concentrations for adenovirus, norovirus GIA, and norovirus GIB were not surveyed in the OC San LRV study.

With respect to DPR-2, matrix spike recovery samples were collected with every other raw wastewater influent sample for a total of 12 matrix spike samples of 24 total samples collected from each WWTP during the study period. In comparison, as described in Chapter 3, a total of 9 raw wastewater matrix spike recovery samples were collected per raw wastewater sampling location over the 24 total samples in the present study (i.e., greater than approximately one in three raw wastewater samples featured a paired matrix spike recovery sample). For each secondary effluent sampling location in the current study, five matrix spike recovery samples were collected, resulting in 20 total secondary effluent matrix spike recovery samples over the course of the study (i.e., greater than approximately one in five secondary effluent samples [20 of 96] featured a paired matrix spike recovery sample). These additional matrix spike recovery samples were deemed appropriate to obtain a higher frequency of recoveries for greater consistency with DPR-2.

For raw wastewater, cultivable enteric virus and coliphage analyses (EPA 1615 and 1602, respectively), DPR-2 used MS coliphage and the phiX174 bacteriophage as viral targets to spike in select samples to evaluate method recovery (matrix spike recovery). In comparison, this study used MS coliphage and poliovirus as viral targets for matrix spikes. Poliovirus was chosen in place of phiX174 because poliovirus shares many physical characteristics to other human pathogens including enterovirus and adenovirus and is therefore a representative surrogate for enteric virus recovery.

6.2 Comparison of Method Recovery Corrections

Native cultivable virus concentration data were corrected using the average cultivable recovery of MS coliphage, SOM coliphage, and poliovirus. Recovery data from all three targets were pooled to calculate a single average recovery value which was then used to correct the native results. Native coliphage concentration data were corrected using the average coliphage recovery of MS coliphage and SOM coliphage. This approach is consistent with method recovery corrections performed for DPR-2 (Pecson et al. 2021). For those targets enumerated using the molecular assay (ddPCR), native concentrations were not recovery-corrected due to the large variability in recovery values as described in Section 3.4.

CHAPTER 7

Recommendations and Future Work for Virus Log Crediting of Wastewater Treatment

Future studies that seek to evaluate enteric virus log removal for wastewater treatment (i.e., secondary or tertiary treated wastewater effluent) can benefit from the strategies used in this study to further develop and execute an efficient microbial monitoring study for log removal crediting. In this chapter, the research team highlights important points that should be considered when developing method quality controls, handling variability of microbial concentration data, and determining the appropriate LRV calculation. These considerations represent the challenges and lessons learned from this particular case (OCWD-OC San study) based on the experience of the project team.

7.1 Virus Recovery Measurements for Quality Control

Measuring recovery via completing matrix spike recovery (MSR) samples is an important part of quality control especially for the target constituent on which a proposed LRV credit is expected to be based. In this study, MSRs were included for one in three raw influent samples or one in five secondary effluent samples (See Section 3.4). Ideally MSRs would be included for every sample collected due to the variability in virus recovery such that each native sample would be corrected by its paired MSR; not having a paired MSR also creates the need to determine how to recovery-correct native samples that do not have a paired MSR sample based on some kind of statistical analysis of the other MSRs. Incorporating MSRs for every sampling event however doubles the study analytical cost. Therefore, MSR samples for this study were scheduled such that each MSR measurement was evenly distributed throughout the sampling calendar for each site. The MSR results were averaged in this study for each major site matrix to determine a single recovery correction factor for that matrix; in other words, despite the fact that recovery was variable for some sampling locations, ultimately an average recovery was used and applied to all native samples to correct for recovery. It may be possible to reduce the variability of the resulting (corrected) microbial concentration dataset by employing additional MSR samples and correcting the native result by its appropriate (paired) MSR. Sample-specific recovery correction could have reduced the high variability in the dataset observed for the present study that resulted in very low LRV at the 5^{th} percentile in the (modified and original) Monte Carlo analysis.

For recovery measurements using molecular methods, more research is needed on the determination of sample recovery for using armored RNA as the matrix spike target for ddPCR. Armored RNA was selected for this study based on its long history of use for quality control purposes in the EPA 1615 method (though not for determining recovery). In this study, the norovirus ddPCR recovery results exhibited major inconsistencies necessitating disregarding the recovery data, while the calculated enterovirus ddPCR recoveries were often negative (i.e., spiked sample had less target than the paired native sample) or near zero. As a result, the study team decided that it was not acceptable to use these recovery data to correct native results

given a minimum acceptance criteria of 5% recovery. Thus, ddPCR native sample results in this study were not corrected by recovery. Should any future studies consider the use of ddPCR for enumeration of multiple virus targets (i.e., enterovirus, norovirus GII, poliovirus as in this study), the study team recommends performing a robust preoptimization study to address any potential issues with sample-matrix interference, detection of armored RNA, and virus recovery.

7.2 Considering the Use of Molecular Methods

While microbial detection via molecular methods (e.g., qPCR, ddPCR) holds promise, there are still many questions around whether removal of gene copy number across treatment is sufficient to assess performance compared to removal of cultivable virus targets given the lack of correlation in concentration between the two methods observed in this and other studies (see Section 5.5). This may be related to the fact that gene copies can include both viable and non-viable virus. If the regulatory authority is unlikely to grant credit based on molecular methods regardless of the value observed compared to cultivable or any other clear criteria, it may be appropriate to exclude these methods/targets in a study aimed specifically at receiving a regulatory credit value in order to save cost and, importantly, reallocate those funds for other needs highlighted in these recommendations (e.g., more MSRs, more frequent sample duplicates, characterize diurnal variability to potentially utilize a residence time-informed sampling scheme, etc.).

7.3 Calculation of Log Removal and How This Informs Sampling Design

This study evaluated microbial concentration data taken from four secondary treatment processes using two statistical approaches to calculate virus log removal. Median LRVs obtained from each approach were similar, suggesting that despite the fundamental differences in analysis, virus removal can be evaluated for each of the treatment processes using both approaches. When considering the use of a conservative estimate of virus removal however, such as using the 5th percentile value of calculated LRVs, the modified Monte Carlo LRVs typically resulted in lower LRVs than the covariance approach. We believe this observation is primarily because of how the modified Monte Carlo simulation randomly pairs raw influent and secondary effluent concentration values that may be similar for certain pairings, producing low LRVs as a result of overlapping (similar) concentrations for a given paired influent and effluent distribution. This only occurs a fraction of the time within the n=10,000 observations, however this estimate is recorded as a possible LRV at the low end of the percentile distribution. Given the regulatory precedent in California for basing credit on the 5th percentile LRV, this may drive the ultimate credit value. Despite the attempt to resolve this issue by censoring data to remove negative LRVs, the study team and GWRS IAP believed that this estimation approach was not representative of the actual low-end virus log removal observed for all four treatment processes (Table 5-2). In other words, the Monte Carlo simulation approach (with or without censoring) may be overly conservative and not reflective of the true low-end plant performance for virus removal. More work is needed to confirm this and to continue to identify superior statistical and/or sampling methods.

In contrast, the covariance approach generally had higher 5th percentile LRVs for microbial targets compared to the modified Monte Carlo and underscores the underlying differences

between each approach. This study proposed a log removal value to California regulators based on a covariance analysis approach of the cultivable virus dataset, largely due to the fact that this statistical method considers the dependency of the raw influent and secondary effluent distribution (in this approach described by the rank-paired covariance), does not generate negative LRVs, did not require censoring of LRV data, and therefore was deemed to more appropriately quantitate the differences between said distributions. Future work may determine a more meaningful method for determining covariance beyond simple rank-pairing used in this study.

An additional notable study observation that may inform choice of sampling approach and/or LRV calculation method was the higher LRVs for activated sludge over TF or TF/SC (which was observed regardless of which of the two statistical methods was used in this study to calculate LRV). Hence, a (potentially overly) conservative approach such as Monte Carlo analysis may be acceptable to a utility if they utilize activated sludge process depending on how much additional credit is being sought. Overall, treatment processes with lower general performance compared to nitrification/denitrification (NDN) processes will likely result in much lower LRVs. In this study, the 5th percentile LRV for the process with the lowest LRV (OC San Plant No. 1 TF) was ultimately proposed as the credit value (using covariance-based approach), because it represented a conservative choice that avoided the need to amalgamate the LRV datasets of four effluents that blend to serve as influent to GWRS toward a single LRV credit value. However, reliance on strictly the P1 TF process significantly reduced OCWD's proposed overall credit value for wastewater treatment given the much higher observed 5th percentile LRV for AS1 and AS2.

7.3.1 Addressing Sample Variability

The study team believes that the variability in cultivable virus occurrence in both OC San P1 and P2 influent and effluent is likely real given the generally good performance of duplicate samples, as opposed to being a result of analytical performance issues or inherent variability. Nevertheless, an improved study design could include duplicates or triplicates at every sampling event, such that the average used to represent that event may dampen any analytical variability and provide a more accurate measurement. If duplicates/triplicates are very consistent, they could be tapered off as the study continues.

Fundamentally, if a target constituent is highly variable in concentration over time (i.e., over hours), collecting influent and effluent samples that are staggered from one another in time based on the treatment system's average residence time is very important, if paired influent/effluent values from the same day are used to calculate LRV. This is because when removal of a target constituent is calculated using these paired influent/effluent values, any peak observed in the effluent concentration is paired with the corresponding (higher) peak that occurred in the influent (or conversely, low effluent and corresponding low influent concentration are paired). The Monte Carlo method does not require residence-time-paired samples because it assumes no relationship between the influent and effluent distribution and randomly chooses sample pairs from along each distribution of all results across the different days sampled. When the target constituent (in this case cultivable virus) is highly variable in the influent for the different days sampled, as seen in this study, the Monte Carlo distribution will

therefore be quite steep with low LRVs based on random pairings of *unrelated* high-effluent samples with low-influent samples. With the requirement to take the 5th percentile LRV, the proposed credit value will be driven by these cases and be very low.

The use of the 5th percentile assumes that a treatment study has directly measured true removal several times (e.g., measuring in-and-out removal from a straightforward unit process to calculate LRV several times) so that the worst or nearly worst measured *removal* (5th percentile) can be used as a conservative estimate for removal credit value. However, the distribution of LRVs generated by the Monte Carlo simulation from pairing random influent and effluent data is not necessarily the true range of treatment removal. As stated above, this is particularly true if a given target constituent is highly variable in concentration, potentially resulting in an overly conservative estimate of performance at low percentiles. While this approach may be acceptable for activated sludge processes where at least 0.4 to 1.6 virus log credit at 5th percentile is likely attainable, it is especially problematic for non-NDN processes where the proposed credit value will approach zero. Hence, the covariance-based approach was favored in this study since it is a fundamentally different statistical approach that does not randomly pair influent-effluent concentrations.

While the covariance-based approach was ultimately used in the present study to calculate a proposed LRV credit for wastewater treatment (under review by regulator at the time of this report), future research should seek to further develop and advance appropriate statistical and/or sampling techniques for determining conservative (5th percentile) LRVs, based on the knowledge that true virus removal across wastewater treatment is likely significant (e.g., this study found median removals of 2.0+ LRV or 99%+ for activated sludge processes and 1.0 to 1.3 LRV or 90 to 95% for trickling filter-based processes). Methods must consider that concentration data for a target like cultivable virus will be highly variable, which could be addressed through study sampling design (e.g., residence-time paired sampling; MSRs for every sample grab) or by considering the use of a more stable virus indicator such as MS and SOM coliphage instead of cultivable virus, or by developing a more appropriate statistical method. In this study and others, coliphage concentration data are much more stable in both the influent and wastewater effluent samples.

7.3.2 Hydraulic Residence Time and Composite Sampling

Collecting samples that are appropriately staggered in time by the average hydraulic residence time of the process to allow LRVs calculated from residence-time-paired influent/effluent samples may result in more accurate (and potentially higher) log removal estimate. However, this is difficult to do due to the long hydraulic residence times of a wastewater treatment plant, which can be difficult to estimate accurately. Furthermore, it is possible that return flows from sidestreams may also influence final concentration results. Another complication is that the influent sample likely utilizes a simple grab sample (an instantaneous timepoint) versus the effluent sample likely requires on-site ultrafiltration (a multi-hour composite), depending on the microbial target. To attempt to address these challenges, future work could consider focusing on individual unit processes of a wastewater treatment plant expected to demonstrate the highest virus removal, in addition to or instead of sampling for removal from raw influent compared to final secondary or tertiary effluent, because an individual unit process will have a

shorter residence time potentially reducing some of this uncertainty and perhaps the influent variability. It may be possible to optimize the sampling approach so that influent and effluent are both grabs or are both composites. Further, pre-studies could characterize the variability in virus concentration over timescales relevant to the residence time (e.g., if the estimated residence time is some number of hours, determine whether influent/effluent sample grabs actually vary significantly within that time) which could support study design, since lack of variability over relevant timescales would indicate that staggering influent/effluent sample collection by residence time is not necessary. If a study is able to successfully residence-time-pair the influent/effluent samples, an appropriate statistical method will need to be developed to determine a 5^{th} percentile LRV, such as based on simple influent/effluent pairing (using the lowest or 5^{th} percentile observed LRV as proposed credit value) or covariance-based approach with pairing based on each collected sample set (rather than by rank as in the present study).

CHAPTER 8

Novel Online Surrogates to Monitor Reverse Osmosis – Background and Study Approach

Note: Chapters 8 and 9 were reproduced with permission from study co-funder United States Bureau of Reclamation Final Report for Agreement No. R18AC00111 "Novel Online Surrogates to Monitor Reverse Osmosis Performance in Reuse Applications" (Tackaert et al. 2021).

Reverse osmosis (RO) is a widely accepted treatment technology in reuse scenarios. RO serves as a physical barrier to even the smallest pathogens and most dissolved constituents. Past studies have shown that commercial RO membranes can achieve greater than 6 logs of removal for MS2 bacteriophage, a commonly accepted surrogate for enteric virus (DeCarolis et al. 2005, Adham et al. 1997, Jacangelo et al. 2015). However, there is a discrepancy between actual log removals achieved and the pathogen log removal credit awarded by regulatory agencies. Current potable reuse regulations require ongoing performance monitoring of RO systems to demonstrate membrane integrity and to protect public health. However, real-time monitoring of viruses is not possible with current technologies. Therefore, surrogates are used to confirm removal and demonstrate overall RO system integrity. Traditional surrogates include electrical conductivity (EC), which results in up to 1.5 logs of observed removal and therefore credits in brackish water applications including potable reuse, and total organic carbon (TOC), which can result in up to 2 logs of removal credit.

Increasing the log removal credits assigned to the RO system in potable reuse treatment facilities has the following main benefits of (1) increasing confidence, from both the industry and the public, in RO's ability to remove high levels of pathogens, and (2) reducing the burden of pathogen removal credits on the rest of the treatment train, which has significant design implications in terms of operational flexibility, costs, energy use and footprint.

8.1 RO Study Objective

The principal objective of this study was to identify and test naturally occurring surrogates for monitoring RO performance for reuse that can demonstrate greater removal and therefore begin to bridge the gap between actual performance and the awarded pathogen log removal credits. Naturally occurring surrogates are preferable to avoid the complexity and expense of spiking constituents into the feed water to achieve sufficient LRV. The project investigated surrogates at the OCWD AWPF in Fountain Valley, California.

8.2 Problem and Needs

One of the key design criteria for potable reuse facilities is the log removal credits that can be assigned to each treatment process. RO has traditionally been and still is under-credited today due to the lack of an online, near-real time, or daily grab sample-based monitoring strategy to continuously demonstrate membrane and system integrity at levels close to actual pathogen

removals. This under-crediting results in additional infrastructure for treatment processes in order to obtain the total credits required by recycled water facility permits. This is a significant issue for the reuse industry and is getting more attention as the reuse industry begins to regulate and design direct potable reuse (DPR) projects. In California, for example, draft regulations require that DPR facilities must demonstrate at least 20-log reduction for virus (SWRCB DDW 2021), where only approximately 2 logs are currently obtained by the RO treatment process using EC or TOC. Potable reuse is a rapidly growing practice, and trending towards requirements for higher log removal credits as the physical and psychological connection between wastewater and drinking water becomes closer (i.e., DPR facilities).

This study aimed to increase the log removal credits awarded to RO in reuse applications by evaluating promising and new online surrogates. For example, the latest increase in log removal credits was 0.5 logs for facilities switching from EC to TOC. The novel surrogates proposed for investigation in this study have the potential to demonstrate 1 to 1.5 additional logs over what TOC monitoring can provide, which can translate into a major increase in confidence with respect to water quality as well as cost savings with respect to water treatment.

8.3 Purpose of Surrogates

Surrogates are used to demonstrate the ability of an RO system to effectively reject pathogens that are of concern for public health. Since RO is a physical barrier that removes contaminants (chemical or microbial) primarily through size exclusion, and the smallest pathogens of concern are viruses, an ideal surrogate must be conservative relative to virus rejection. In addition, an ideal surrogate accurately tracks decreases in virus rejection that may occur if there are compromises or breaches in the RO system. In the context of RO integrity monitoring, a surrogate for virus is not intended to simulate an actual virus (i.e., have the same size, structure, behavior, properties, etc.), but rather to accurately indicate that the RO system is functioning normally (high integrity) and whether any system breach has occurred as indicated by reduced log removal of the surrogate which might in some cases correspond to decreased removal of actual virus.

Figure 8-1 contrasts the behavior of two theoretical surrogates – poor and good – versus virus rejection with respect to different degrees of compromise. The good surrogate remained conservative to virus rejection during a “no compromise” condition as well as when compromises of different degrees were imposed. In contrast, while the poor surrogate did remain conservative to virus during the “no compromise” condition, it failed to accurately track the decrease in virus rejection, ultimately overestimating virus rejection itself for a “severe compromise” condition.

In addition to these characteristics, an even better surrogate for RO integrity monitoring demonstrates an overall high degree of log removal, i.e., a high LRV greater than 1.5 (EC) or 2 (TOC) that ideally approximates pathogen rejection yet remains conservative, in order to bridge the gap between actual performance of RO (> ~6 LRV for real virus) and the awarded pathogen log removal credit (currently ~ 2 LRV).

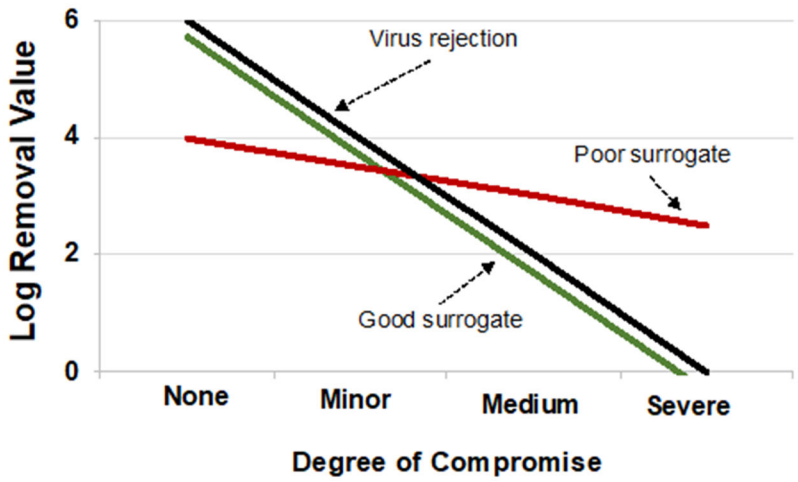


Figure 8-1. Response of Theoretical Surrogates to Membrane Compromises in Comparison to Virus Removal.
Source: Adapted from Trussell Technologies 2017a.

8.4 Regulatory Framework for Surrogates in Reuse

Per current California indirect potable reuse regulations and draft DPR regulations, the pursuing agency must validate each treatment process and provide evidence of the treatment process's ability to reliably and consistently achieve the log reduction pursued for the process, which can be done using a challenge test approved by the State Board (22 CCR § 60320.208; 22 CCR § 60320.308; SWRCP DDW 2021). Specific to the RO treatment process, monitoring should include one form of continuous monitoring, as well as an associated surrogate and/or operational parameter limits and alarm settings that indicate when the integrity has been compromised (22 CCR § 60320.201; 22 CCR § 60320.302). The California DDW has approved EC and TOC as acceptable surrogates for pathogen LRV credit for RO systems (22 CCR § 60320.302). Other surrogates, such as strontium which currently does not have an available online method, have since been proposed (e.g., City of San Diego 2019 – T22 ER) using a tiered approach that includes EC and TOC as backup surrogates.

Additional regulatory guidance for monitoring the integrity of RO systems is provided in the US Environmental Protection Agency's Membrane Filtration Guidance Manual (MFGM) (EPA 2005). Importantly, the MFGM is expressly directed toward membrane filtration systems seeking to gain *Cryptosporidium* removal credit that is compliant with the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). As such, the MFGM is geared toward membrane systems, including RO, that fall under drinking water regulations. Nonetheless, there are important concepts from the MFGM that are also applicable per current indirect potable reuse regulations to define suitable surrogates. Per the MFGM, a membrane system must demonstrate membrane integrity through two separate tests to receive removal credit for pathogens:

1. Periodic direct integrity testing (DIT) and
2. Continuous indirect integrity monitoring (CIIM)

The EPA Membrane Filtration Guidance Manual defines a DIT as a "physical test applied on each membrane unit and monitored on a daily basis in order to identify and/or isolate integrity breaches" and a CIIM as "monitoring of some aspect of the filtrate water quality […] at a

frequency of no less than once every 15 min" such that "a marked decline in filtrate quality may indicate an integrity problem." (EPA 2005). In this context, "some aspect" of water quality is interpreted as either a bulk surrogate, such as TDS, EC, and TOC, or a molecular marker such as neutral organic and inorganic molecules, and ions.

With the lack of an equivalent for the pressure decay test utilized effectively for low-pressure membrane systems (i.e., micro-, ultra-filtration) and classified as a "physical test," there is currently no such equivalent DIT method for RO. Per the MFGM, the confirmation of integrity using molecular markers can be performed in lieu of a pressure-decay test. As such, regulators have approved online EC and TOC for pathogen LRV credit based on the concept of molecular markers from the MFGM (22 CCR § 60320.302, Bernados 2018). Thus, RO membrane integrity at potable reuse facilities is demonstrated through the monitoring of TOC and/or EC in the feed and permeate.

8.5 Technical Approach and Methods

This section describes the test site, sampling approach, and surrogates selected for monitoring.

8.5.1 OCWD RO Facility Test Site

The evaluation of naturally occurring surrogates for monitoring RO performance was performed onsite at OCWD's AWPF in Fountain Valley, California. The GWRS AWPF facility treats secondary-treated wastewater to produce 100 MGD of highly purified water that would otherwise be discharged to the ocean. The treatment train is comprised of microfiltration (MF), RO, ultraviolet disinfection and hydrogen peroxide addition (UV/H_2O_2) as an advanced oxidation process (UV/AOP), followed by decarbonation and lime stabilization Figure 8-2. Chlorine is added before MF to form chloramines, and antiscalant and sulfuric acid are added before RO to control scaling.

The AWPF RO process uses three types of membranes, Hydranautics ESPA2-LD, Dupont FilmTec BW30XFRLE and LG BW400EX in standard 8-inch pressure vessels in different 5-MGD units depending on year of replacement need and bids received. All membranes are thin film polyamide membranes with high flux and high salt rejection. There is a total of 21 RO treatment units at the facility, each with 5-MGD rated capacity, running in parallel to produce the total 100 MGD of RO permeate (one unit is redundant). Each 5-MGD unit features three stages and operates over a range of total recovery from 80 to 85%. The finished water is recharged into the local groundwater aquifer (a drinking water source), as opposed to being directly used for drinking water distribution system (i.e., delivery straight to tap, DPR).

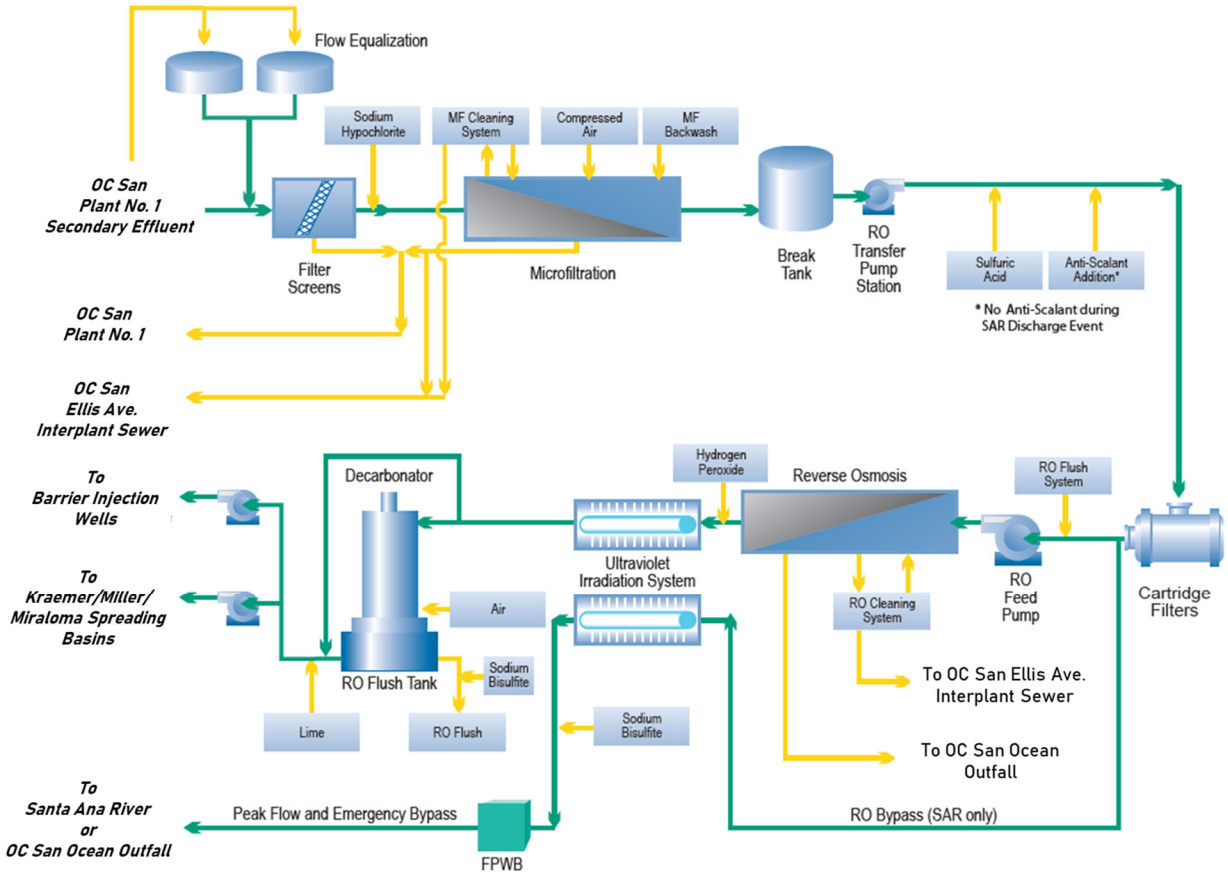


Figure 8-2. OCWD AWPF GWRS Treatment Train.
Source: Tackaert et al. 2021.

The selected test surrogates for this study were primarily monitored on one of the full-scale 5-MGD RO units equipped with the Hydranautics ESPA2-LD RO membranes and operated at 85% recovery (Figure 8-3). For test surrogates with available online instruments (free ATP and fluorescence), the online instrumentation was installed on the feed and permeate sides of the monitored RO unit. Grab samples for surrogates without online instrumentation (strontium and sulfate) were collected using ISCO 3700 portable auto samplers to sample the feed and permeate from the monitored 5-MGD RO unit at preselected time intervals. Although online instrumentation for strontium and sulfate were not available when the study was initially planned, online instruments are now available commercially (separate instrument for strontium versus sulfate) such that findings from this study's grab samples (i.e., LRVs) can be assumed to be representative of LRVs that would also be achieved from online monitoring given that these instruments' manufacturer-reported detection limits appear to be sufficient for RO feed and permeate. For nanoparticles, grab samples were collected and analyzed with a bench-top unit, recognizing that an online version of the instrument could potentially be developed should bench data be promising, based on comments from the manufacturer.

Figure 8-3. A 5-MGD RO Unit at OCWD AWPF Equipped with Hydranautics ESPA2-LD Membranes.
Source: Tackaert et al. 2021.

8.5.2 Selection of Surrogates

Based on a literature review and OCWD historical water quality data query, five (5) surrogates were selected for monitoring and comparison during this study. Two of the novel surrogates, free ATP and nanoparticles have not been previously evaluated. These candidates were identified by OCWD based on prior use of free ATP (via grab samples) for general water quality purposes in the AWPF (e.g., monitoring cartridge filter biogrowth ahead of RO), and knowledge that an online ATP analyzer measuring both free and cellular ATP had become available just before this study began, and OCWD use of online nanoparticle measurement in a 2016-2019 research study focused on MF pre-coagulation (Rajagopalan et al. 2021). The other surrogates selected for monitoring were fluorescence Peak C, strontium and sulfate. Background on each surrogate is presented below.

All selected surrogates are naturally occurring in treated municipal wastewater and in the RO feed water at OCWD. No non-native surrogates were selected since the focus of this study was naturally occurring surrogates to avoid spiking compounds due to cost and operational complexity of such an approach. Four of the surrogates, free ATP, fluorescence Peak C, strontium and sulfate, were monitored for 3 to 6 months at one of OCWD's full-scale 5-MGD RO units to assess their feasibility to replace traditional surrogates (TOC and EC) (Table 8-1). For the fifth surrogate, nanoparticles, bench-scale nanoparticle analysis indicated that current instrumentation and technology is not sensitive enough to detect enough nanoparticles for LRV purposes and/or the nanoparticle concentration in the RO feedwater and RO permeate is too low. Therefore, nanoparticles were not monitored for the remainder of the study.

Table 8-1. Summary of Five Surrogates Monitored at OCWD.

Parameter/ Surrogate	Free ATP	Fluorescence Peak C	Strontium	Sulfate	Nanoparticles
Sampling Mode	Online	Online and grab samples	Grab samples	Grab Samples	Grab samples
Frequency	30 minutes	every second (averaged)	hourly for 24 hours	Hourly for 24 hours	NA
Monitoring Location	RO feed and RO permeate from same 5-MGD RO unit for all surrogates				
Analyzer/ Method	Hach EZ7300 ATP	Turner Cyclops 7	EPA 200.8	EPA 300.0	Particle-Metrix (ZetaView Multiple Parameters Particle Tracking Analyzer)
Detection limit	0.5 pg/L	0.1 µg/L	0.3 µg/L	0.25 mg/L	10^6 nanoparticles/mL

NA = not applicable
Source: Tackaert et al. 2021.

8.5.3 ATP

ATP is a nucleotide found in all living cells and is therefore useful as a surrogate measure of all active and unculturable microbial cells in a water sample. An ATP measurement provides a better estimate of the total microbial biomass than heterotrophic plate counts, where only a fraction of the total cells can be quantified (Maki et al. 1986, Siebel et al. 2008). The total ATP in a water sample is the sum of cellular ATP (cATP) that is still bound within the living cells plus extra-cellular ATP (free ATP) present in water from dead or lysed cells. ATP's relatively high molecular weight (507 Da) and abundance in the RO feed water made free ATP a potential candidate for use in RO integrity monitoring. Free ATP should be removed by RO membranes which typically reject compounds greater than 150 Da (Ozaki and Li 2002). Some studies have shown that the molecular weight cut-off for certain low-pressure RO membranes, such as the ones used in the AWPF, are closer to 220 Da (Kimura et al. 2003).

For this study, two online ATP-based detection systems, Hach EZ7300-ATP online analyzer, which measures both cATP and free ATP were installed onto one of the AWPF's 21 5-MGD RO units. Initially, two online EZ-ATP analyzers were installed, one on the RO feed and one on RO permeate. Each instrument was calibrated using ATP standard solutions (Promega Corp). Later, the two EZ-ATP analyzers were replaced with a new dual stream EZ-ATP analyzer that project partner Hach developed for this study, capable of analyzing both RO feed and RO permeate in one instrument. Free ATP measurements were collected every 7 to 30 minutes with a detection limit of 0.5 pg/L.

8.5.4 Fluorescence Peak C (Humic-Like fDOM)

Fluorescence spectroscopy has shown promise as a sensitive and accurate monitoring tool for water quality and process performance, in part because it is a rapid and fairly inexpensive analytical technique that requires no reagents or sample pretreatment. Fluorescence spectroscopy has been identified by the industry as having strong potential for online monitoring of recycled water quality and treatment process performance because it can distinguish between different types of organic carbon and has been shown to be 10 to 1000

times more sensitive than other commonly used techniques such as UV absorption spectroscopy and high-performance liquid chromatography (HPLC) (Henderson et al. 2009). A previous study by Singh et al. (2012) identified humic-like fDOM (Peak C) in RO permeate as having the greatest potential for evaluating membrane integrity compared to other fDOM fluorescence peaks.

Fluorescing dissolved organic matter (fDOM) is present in wastewater and has a characteristic fluorescence pattern. Fluorescence spectroscopy offers insight into both the quantity and composition of fDOM. The following are key fluorescence signals of fDOM that have been used to distinguish treated wastewater effluents: Humic-like (Peak A: $\lambda_{Ex/Em}$ = 237-260/400-500 nm; Peak C: $\lambda_{Ex/Em}$ = 320-340/410-430 nm and Peak C_2: $\lambda_{Ex/Em}$ = 370-390/460-480 nm), tyrosine-like (Peak B_1: $\lambda_{Ex/Em}$ = 225-237/30-321nm and Peak B_2: $\lambda_{Ex/Em}$ = 248/310 nm) and tryptophan-like (Peak T_1: $\lambda_{Ex/Em}$ = 275-290/340-360 nm peaks and Peak T_2: $\lambda_{Ex/Em}$ = 225-237/340-381 nm).

Initially, analysis of RO feed and RO permeate grab samples for humic-like Peak C was performed using an Aqualog benchtop fluorometer from Horiba Scientific (Tokyo, Japan) for an excitation range 240-470 nm and emission range of 280-580 nm. Aqualog supplied software was used to collect fluorescence spectra and processed using a modified Fluorescence Regional Integration (FRI) (Stanford et al. 2011; Gerrity et al. 2011) and the Fluorescence Index (FI) (McKnight et al. 2001). The excitation-emission matrix (EEM) data were corrected for the Raman Scatter by subtracting emission of the blank and corrected for inner-filter effect (MacDonald et al. 1997). Peak C fluorescence signature of the RO feed and permeate were extracted and an average LRV of 2.51 was calculated (Figure 8-4). Following this grab-sample based validation step where it was confirmed that humic Peak C rejection across the RO membrane could be measured, two Cyclops 7 fluorometers from Turner Designs (San Jose, CA) were purchased to monitor online fluorescence in the feed and permeate to determine diurnal fluctuations and long-term variability for LRV monitoring. The Cyclops 7 fluorometers have a Peak C detection limit of 0.5 µg/L.

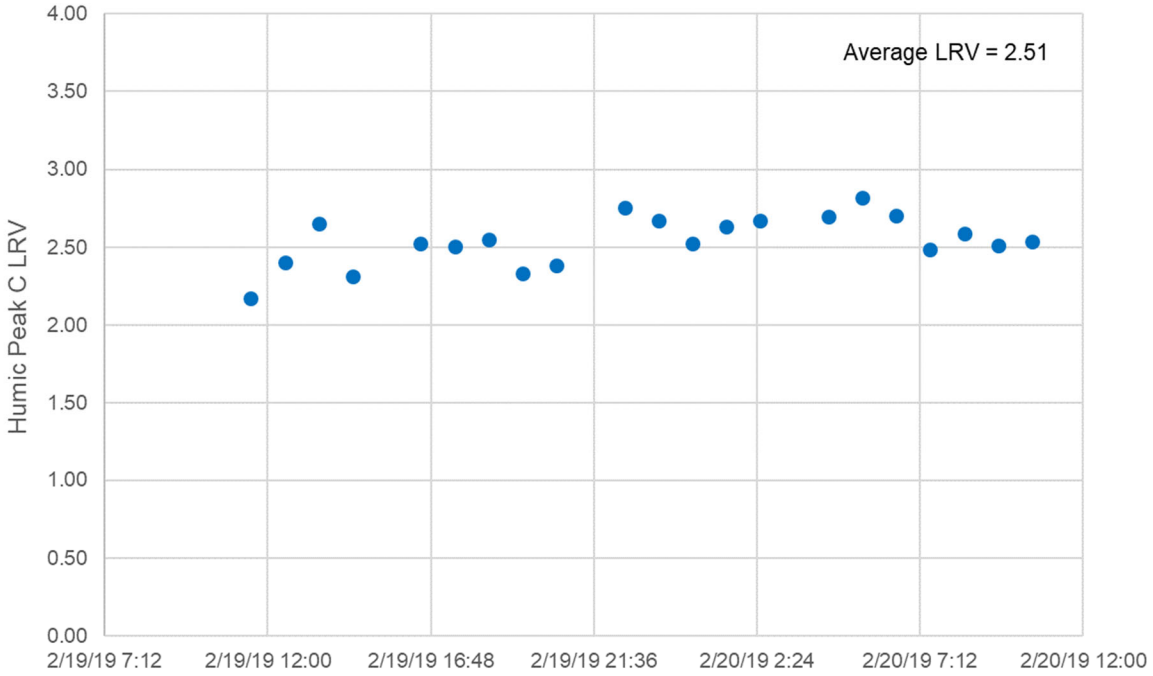


Figure 8-4. Fluorescence Peak C Rejection across a 5-MGD RO Unit with ESPA2-LD RO Membranes Measured Using Horiba Scientific Aqualog Benchtop Fluorometer.
Grab samples collected every hour for 24 hours.
Source: Tackaert et al. 2021.

8.5.5 Naturally Occurring Ions: Sulfate and Strontium

Similar to ATP, sulfate occurs naturally in treated municipal wastewater and is present at the RO feed water for advanced treatment such that there is no need to "spike" these constituents into the feed. Therefore, sulfate has been previously identified as a potential surrogate for monitoring membrane integrity. A study by Kruithof et al. 2001, observed sulfate reduction from 140 mg/L in the feed to 0.1 mg/L in the permeate, which is a log removal value above 3. In the case of OCWD's facility, it is present in AWPF feedwater at 150 to 250 mg/L, which is high enough to quantify significant removal across the RO membranes. Online sulfate measurement can be performed by ion chromatography (IC), but the system is relatively expensive and should be assessed to validate use for this application. An alternative method of analysis for monitoring RO integrity is via grab sampling and performing same-day analysis. Even with grab samples, frequency of samples can be high enough to collect adequate data to determine log removal and diurnal fluctuations.

Strontium also occurs naturally in the RO feed water. Its salts are detectable in essentially all drinking waters. In the case of OCWD's facility, it is present in AWPF feedwater at 650 to 750 µg/L, which is high enough to quantify significant removal across the RO membranes. Online strontium measurement can also be performed but like for sulfate, this system is relatively expensive and should be assessed for validation for this application. Alternatively, as stated above for sulfate, the frequency of grab samples can be high enough to collect adequate data to determine log removal and diurnal fluctuations.

Sulfate and strontium grab samples were collected using ISCO 3700 portable auto samplers. Ion analysis was performed by a subcontracted laboratory (Eurofins Eaton Analytical – Monrovia, CA). Sulfate was analyzed using EPA method 300.0 with a method reporting limit (MRL) of 0.25 mg/L (instrumentation: ion chromatography). Strontium was analyzed using EPA method 200.8 with a MRL of 0.30 µg/L (instrumentation: inductively coupled plasma – mass spectroscopy [ICP-MS]).

Testing for sulfate and strontium focused on five sampling events and took place over a 2-year period. Each sampling event featured multiple, sequential grab samples to understand potential for diurnal variation and to simulate data that would be observed with an online instrument. Four of the sampling events (03/12/2019, 06/12/2019, 02/24/2020, 09/26/2020) occurred when older ESPA2-LD membranes were still installed in the sampled full-scale RO unit and one sampling event (12/20/2020) was performed after new Dupont FilmTec BW30XFRLE were installed. The membrane change was not driven by this study and was performed as part of normal plant maintenance and operations.

8.5.6 Nanoparticles

The use of nanoparticles as a novel surrogate show promise in the field of RO integrity monitoring as a nanoparticle analyzer could be used to measure viral sized particles in the RO feed and permeate. Prior work has evaluated use of silver nanoparticles (Antony et al. 2014), but this would require spiking with such particles.

A nanoparticle tracking analyzer (NTA) was evaluated for this study to measure viral sized particles in the RO feed and permeate. The theory of NTA is that particles in suspension are under Brownian motion and the speed of the particle is in reverse proportion to their size. Illuminating the particles with a laser (photon) causes light intensity fluctuations, and the fluctuating rates (Brownian motion) are recorded in a video. The measured change in location within a certain time(t) gives a specific diffusion coefficient (D) for each individual particle. Using the Stoke-Einstein equation, the hydrodynamic particle radius (r) and thus the diameter of each particle is calculated (www.particle-metrix.de). Nanoparticle tracking is a rapidly evolving field and is expected to offer online monitoring capabilities in the near future. Thus, this may allow further evaluation of nanoparticles as a potential surrogate for RO integrity monitoring beyond the initial findings from the present study.

For this study, nanoparticle monitoring was performed using a ZetaView Particle Metrix Analyzer by Particle Metrix (Wildmoos, Germany). RO feed and RO permeate grab samples were collected and analyzed using the Particle Metrix analyzer. Depending on the sample type and measuring mode, the analyzer has a measuring range for particle sizes between 0.015 µm and 5 µm. The Analyzer is able to measure both size and concentration of particles.

8.5.7 LRV Calculation Methodology

Constituent removal efficiency by the RO process (calculated as a log removal value, or LRV) for each constituent was calculated using an equation from the EPA Membrane Filtration Guidance Manual (EPA 2005):

$$\boldsymbol{LRV = log(C_f) - log(C_p)} \quad \text{(Equation 8-1)}$$

where:

LRV = log removal value demonstrated during sampling of constituent

C_f = feed concentration of constituent

C_P = filtrate concentration of constituent

For OCWD LRV calculations, it takes approximately 2 minutes for a plug of water to move through a 5-MGD RO unit (RO feed to RO permeate). Thus, RO feed and permeate concentrations were measured two minutes apart via an online instrument (or grabs) located at the feed and permeate, and these paired data were used to calculate the LRV for each time point. However, this staggered approach is likely unnecessary given that the feed and permeate concentrations do not change appreciably in such a short time.

It should be noted that any averages of LRVs over time were taken by averaging the percent removal data (e.g., 90% not 1-log form of the data) and then converting the average percent removal value to a log removal value. It is a common error to take arithmetic averages of log values and it is not mathematically correct (Schmidt et al. 2020). The error results in overestimation of log removal. When the log values do not vary substantially, the error is less significant.

CHAPTER 9

RO Monitoring Results and Discussion

Note: Chapters 8 and 9 were reproduced with permission from study co-funder United States Bureau of Reclamation Final Report for Agreement No. R18AC00111 "Novel Online Surrogates to Monitor Reverse Osmosis Performance in Reuse Applications" (Tackaert et al. 2021).

This chapter presents the LRV findings for each measured surrogate from RO monitoring and next steps for OCWD seeking enhanced log removal RO credit for virus.

9.1 ATP

Free ATP measurements were collected every 30 minutes over a 2-month period using the dual stream EZ-ATP analyzer. Instantaneous LRV ranged between 2.60 to 3.30 with an average LRV of 3.03 (Figure 9-1). The gaps in data collection shown in the figure were due to instrument scheduled maintenance and the later gap in 2020 was related to the SARS-CoV-2 pandemic.

Diurnal variability in the RO feed was observed with higher free ATP concentrations between 11 AM to 3 PM, and the diurnal variation propagated to the RO permeate. The LRV increased as the free ATP increased in the RO feed. The diurnal variation may be the result of varying flows and water quality from the upstream wastewater treatment facility. Even with increased RO feed concentrations, with only few exceptions, the RO permeate free ATP remained ≤1.7 pg/mL. The EZ-ATP analyzer reports values below its reported free ATP detection limit of 0.5 pg/mL. If observed permeate values were below the free ATP detection limit of 0.5 pg/mL, the detection limit value was used to calculated LRV. This is a conservative approach since the true LRV may be greater if lower detection limits were to be used.

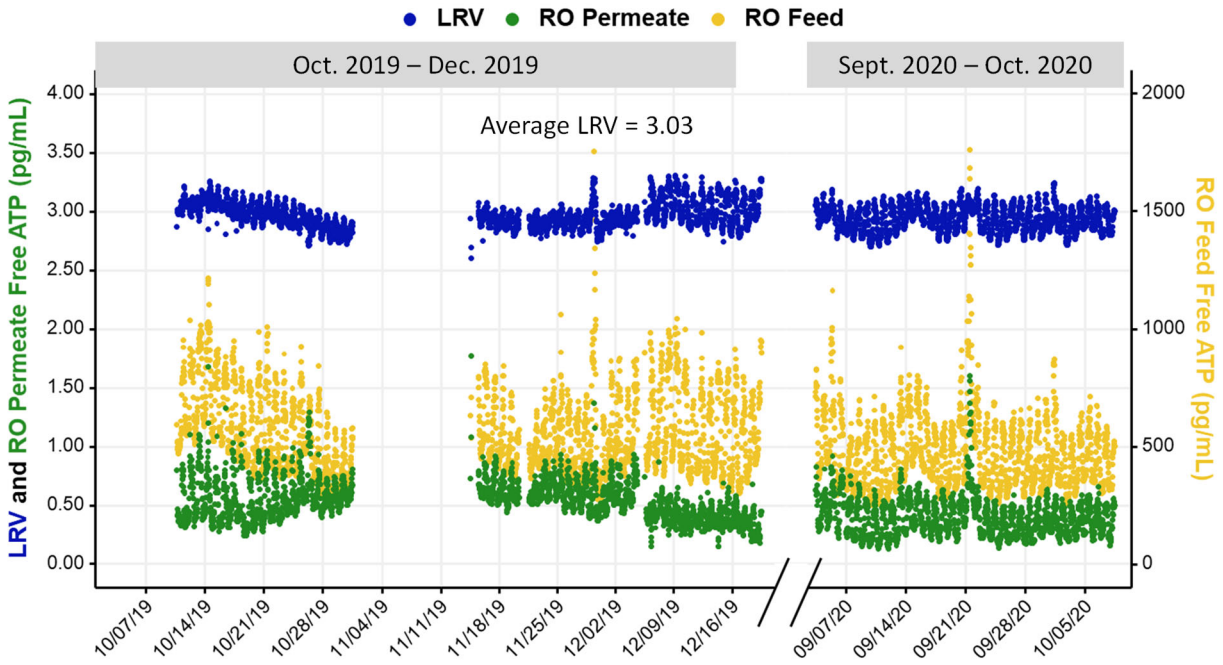


Figure 9-1. Free ATP Rejection as LRV (Blue) across a 5-MGD RO Unit with ESPA2-LD RO Membranes Measured Using Dual Stream ATP Analyzer.
RO feed concentration of free ATP (yellow) and permeate concentration (green) are also shown.
Source: Tackaert et al. 2021.

9.2 Fluorescence Peak C (Humic-Like fDOM)

Peak C fluorescence data was gathered using online fluorometers, measured in the RO feed and permeate to determine diurnal fluctuations and long-term variability for LRV monitoring (Figure 9-2). Each graphed data point in the Figure 9-2 represents a 15-minute average of 1 second measurements. Online Peak C fluorescence LRV of ESPA2-LD membranes ranged between 2.27 to 3.00 with an average of 2.70. The gap in data collection shown in the figure was due to computer and acquisition software issues. As with free ATP, diurnal variability in the RO feed was observed with higher Peak C concentrations between 11 AM to 3 PM, and the diurnal variation propagated to the RO permeate. The LRV increased as the Peak C increased in the RO feed. The diurnal variation may be the result of varying flows and water quality from the upstream wastewater treatment facility.

Online Peak C fluorescence was measured for 24 hours after the new Dupont FilmTec BW30XFRLE membranes were installed (Figure 9-3). The 24-hr online sampling event was performed in parallel to 24-hr strontium and sulfate sampling events on 12/22/2020 (described below in Section 9.3). The DuPont membrane Peak C fluorescence LRV ranged between 2.95 to 3.05 with an average of 2.99 (Figure 9-3), which is slightly higher than what was measured previously with ESPA2-LD membranes. On this day, the RO feed diurnal variability was lower between 11 AM and 3 PM, opposite of what was observed previously (Figure 9-2). As stated above, the change in RO feedwater is the result of varying flows and water quality from the upstream wastewater treatment facility (OC San). Because the diurnal variation propagates to the RO permeate the overall LRV remained >2.9.

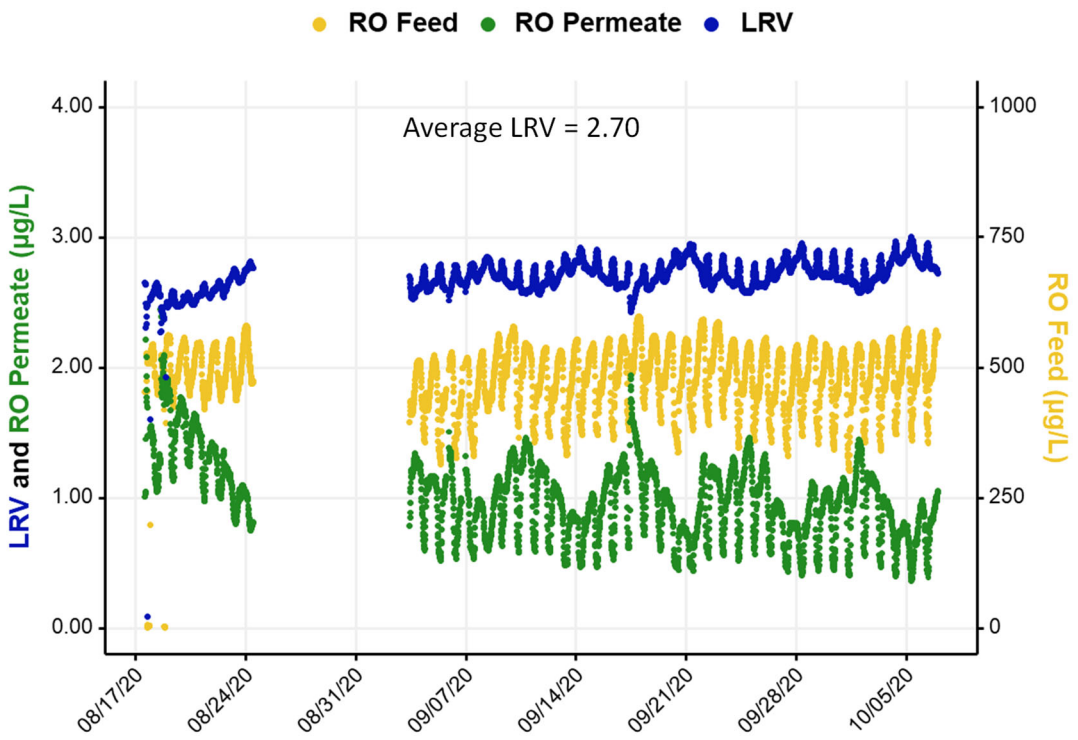


Figure 9-2. Fluorescence Peak C Rejection (Blue) across a 5-MGD RO Unit with ESPA2-LD RO Membranes Measured Using Online C3 Fluorometers.

Each graphed data point represents 15-minute average of 1 second measurements. RO feed concentration of fluorescence Peak C (yellow) and permeate concentration (green) are also shown.

Source: Tackaert et al. 2021.

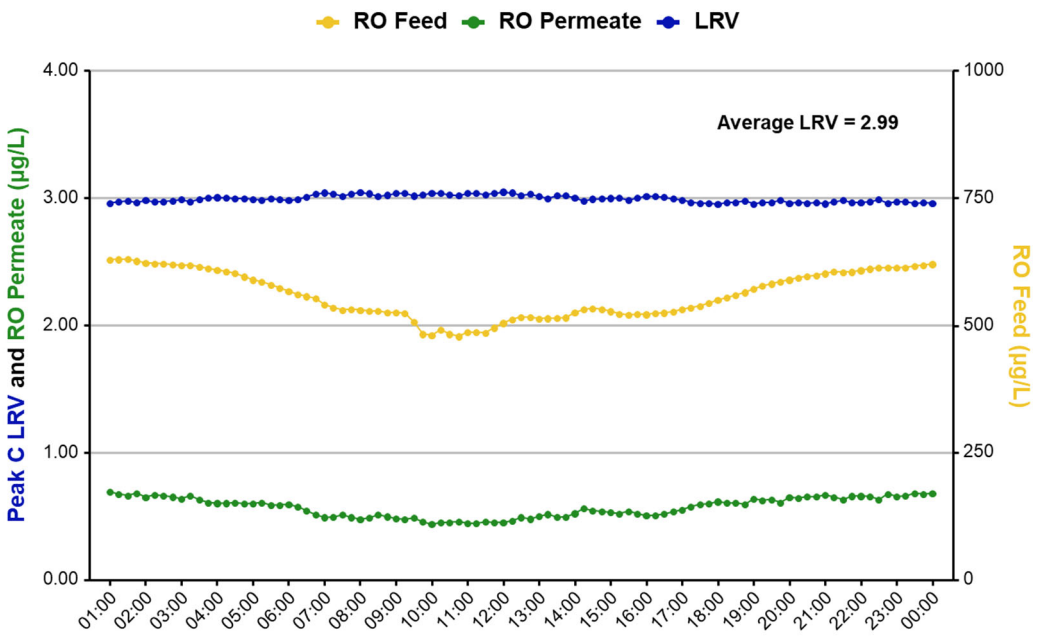


Figure 9-3. Fluorescence Peak C Rejection (Blue) across a 5-MGD RO Unit with Dupont FilmTec BW30XFRLE Membranes.

Each graphed data point represents 15-minute average of 1 second measurements. Measurements collected for 24 hours. RO feed concentration of fluorescence Peak C (yellow) and permeate concentration (green) are also shown.

Source: Tackaert et al. 2021.

9.3 Naturally Occurring Ions: Sulfate and Strontium

Compared to the diurnal variability in LRV observed for free ATP and fluorescence Peak C, both sulfate and strontium LRVs were extremely stable with LRV maintained in a narrow, steady range. Data shown in Figure 9-4 (for sulfate) and Figure 9-5 (for strontium) represents five sampling events performed on 03/12/2019 (ESPA2-LD), 06/12/2019 (ESPA2-LD), 02/24/2020 (ESPA2-LD), 09/26/2020 (ESPA2-LD) and 12/22/2020 (Dupont FilmTec BW30XFRLE) with grab samples taken every hour for 24 hours. For the last sampling event, new RO membranes had been installed in the sampled RO unit approximately two months prior. LRVs measured from additional grab samples from the RO unit after the Dupont membranes were installed are shown in Table 9-1 (sulfate and strontium). Table 9-2 (strontium) and Table 9-3 (sulfate) show additional monthly/bimonthly grab samples collected later from other OCWD RO units in the RO facility (i.e., other membrane types from different manufacturers).

Sulfate (Figure 9-4) showed a slightly lower LRV than strontium (Figure 9-5). Average daily sulfate LRVs on these different days (sampling events) were 2.97 (Figure 9-4A), 2.85 (Figure 9-4B), 2.89 (Figure 9-4C), 2.92 (Figure 9-4D) and 2.94 (Figure 9-4E). Average LRV for all 5 sampling events was 2.91. There was no apparent difference in LRVs between the ESPA2-LD and DuPont membranes. Beyond this more intensive (hourly) sampling, the additional monthly/bimonthly grab samples that were collected later from the same 5-MGD RO unit for comparison over time showed sulfate LRVs eventually improved to >3 (Table 9-3). RO permeate mostly remained at or below the MRL value of 0.25 mg/L, which was used to calculate LRV when the sample value

measured less than 0.25 mg/L. Note that open green squares on Figure 9-4 represent permeate concentrations less than the MRL for sulfate.

As was observed for sulfate, strontium LRV appeared to be very stable and did not exhibit the same diurnal fluctuations as free ATP and fluorescence Peak C. Strontium consistently provided a detectable LRV with average daily LRVs of 3.29 (Figure 9-5A), 3.24 (Figure 9-5B), 3.24 (Figure 9-5C) and 3.14 (Figure 9-5D) for the sampling events corresponding to ESPA2-LD membranes. A lower LRV of 2.86 (Figure 9-5E) was observed after the new DuPont membranes were installed in this RO unit; since this was not observed for sulfate, this indicates that strontium may be a more sensitive surrogate for detecting minor decreases in rejection or integrity. Findings from these sampling events show strontium rejection was more stable with ESPA2-LD membranes than the new Dupont FilmTec BW30XFRLE. It should be noted that newly installed RO membranes may take up to several weeks to acclimate and to stabilize. During the acclimation period membrane rejection of some constituents may vary but eventually stabilizes to produce a more consistent permeate. Based on the additional monthly/bimonthly grab samples collected later on, LRVs stabilized and improved to >3 (Table 9-2). The MRL value was used to calculate LRV when the sample value measured less than 0.30 µg/L. Note that open green circles on Figure 9-5 represents permeate concentrations less than the MRL for strontium.

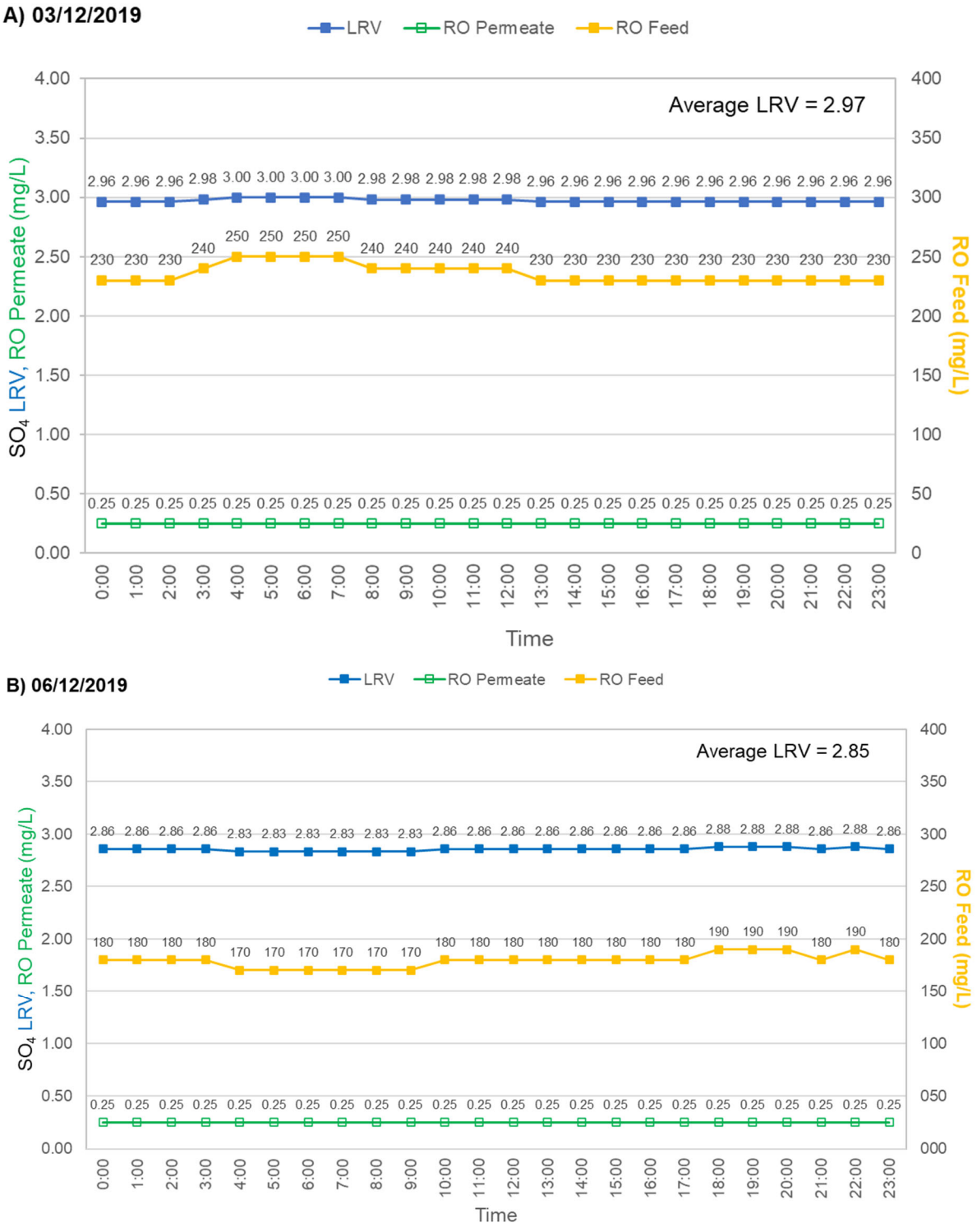
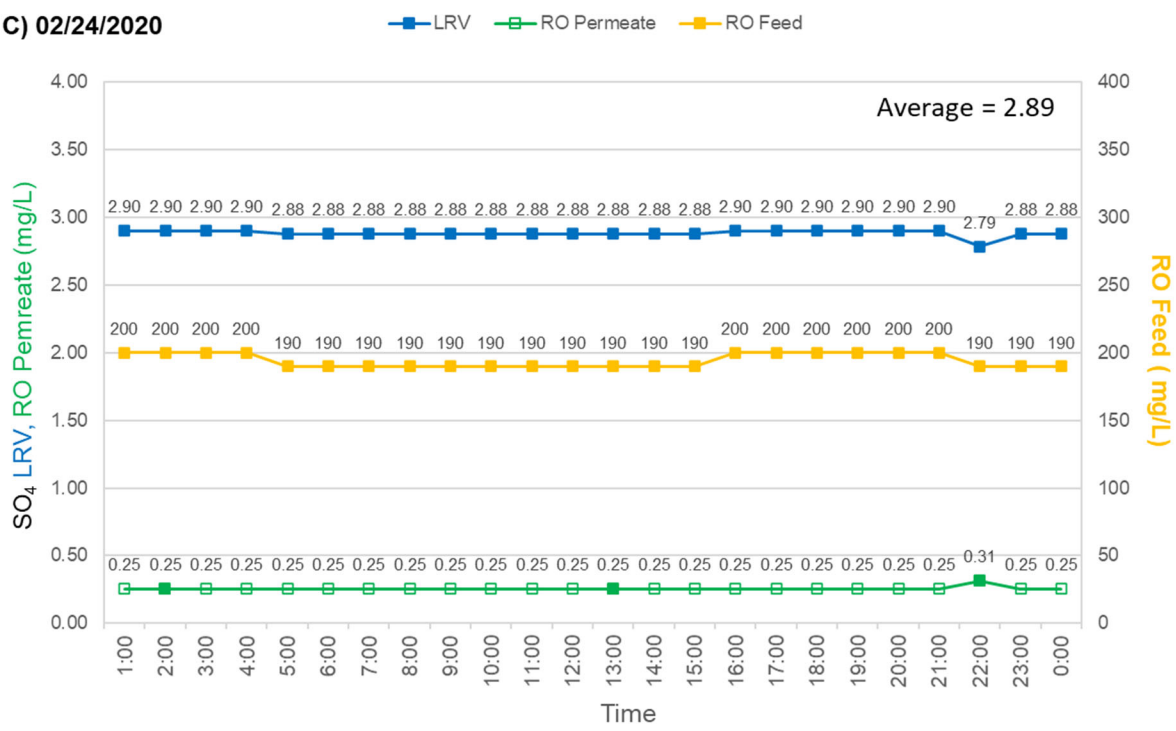


Figure 9-4. Sulfate Rejection (Blue) across a 5-MGD RO Unit.

Charts A through D represent sulfate removal with ESPA2-LD RO membranes. Chart E* represents sulfate removal with Dupont FilmTec BW30XFRLE, installed 10/31/2020. RO feed concentration of sulfate (yellow) and permeate concentration (green) are also shown.

Source: Tackaert et al. 2021.

(continued)



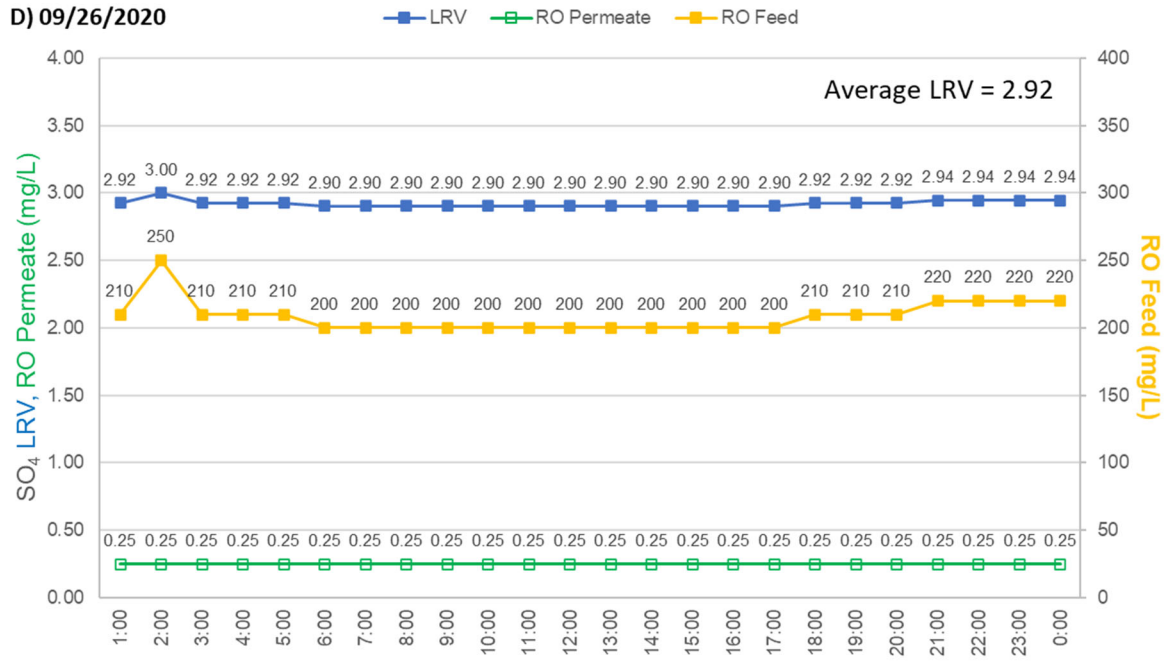


Figure 9-4. Continued.

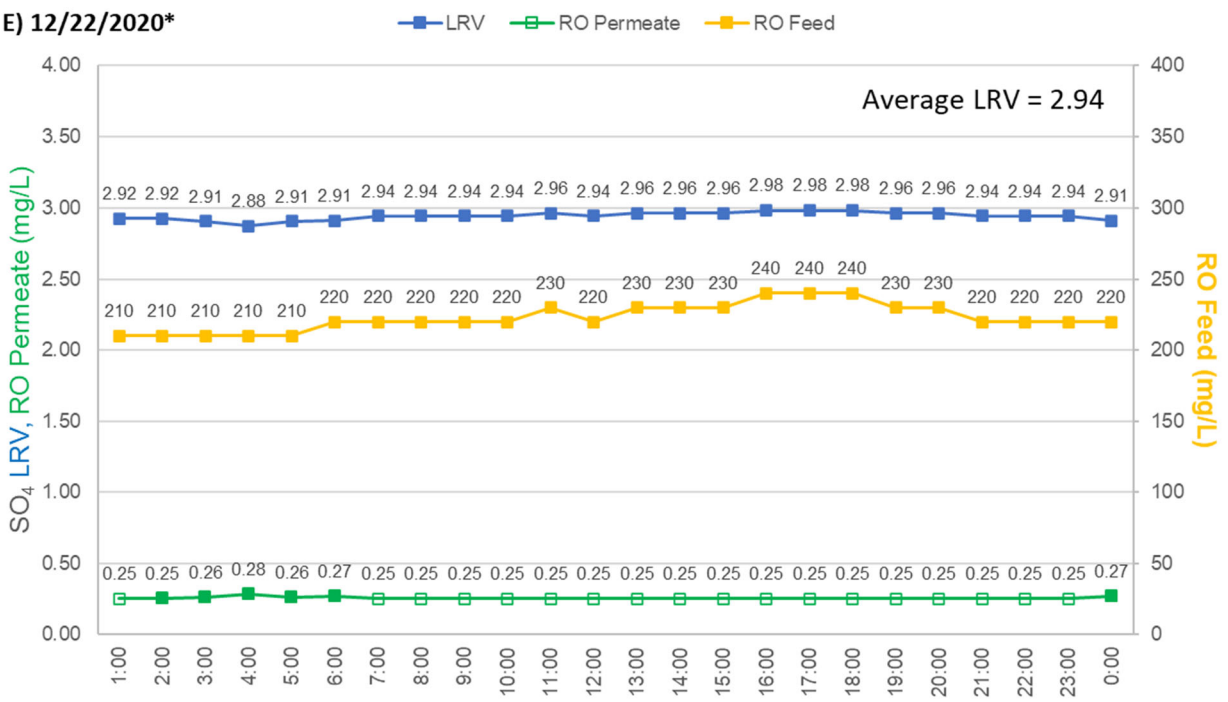


Figure 9-4. Continued.

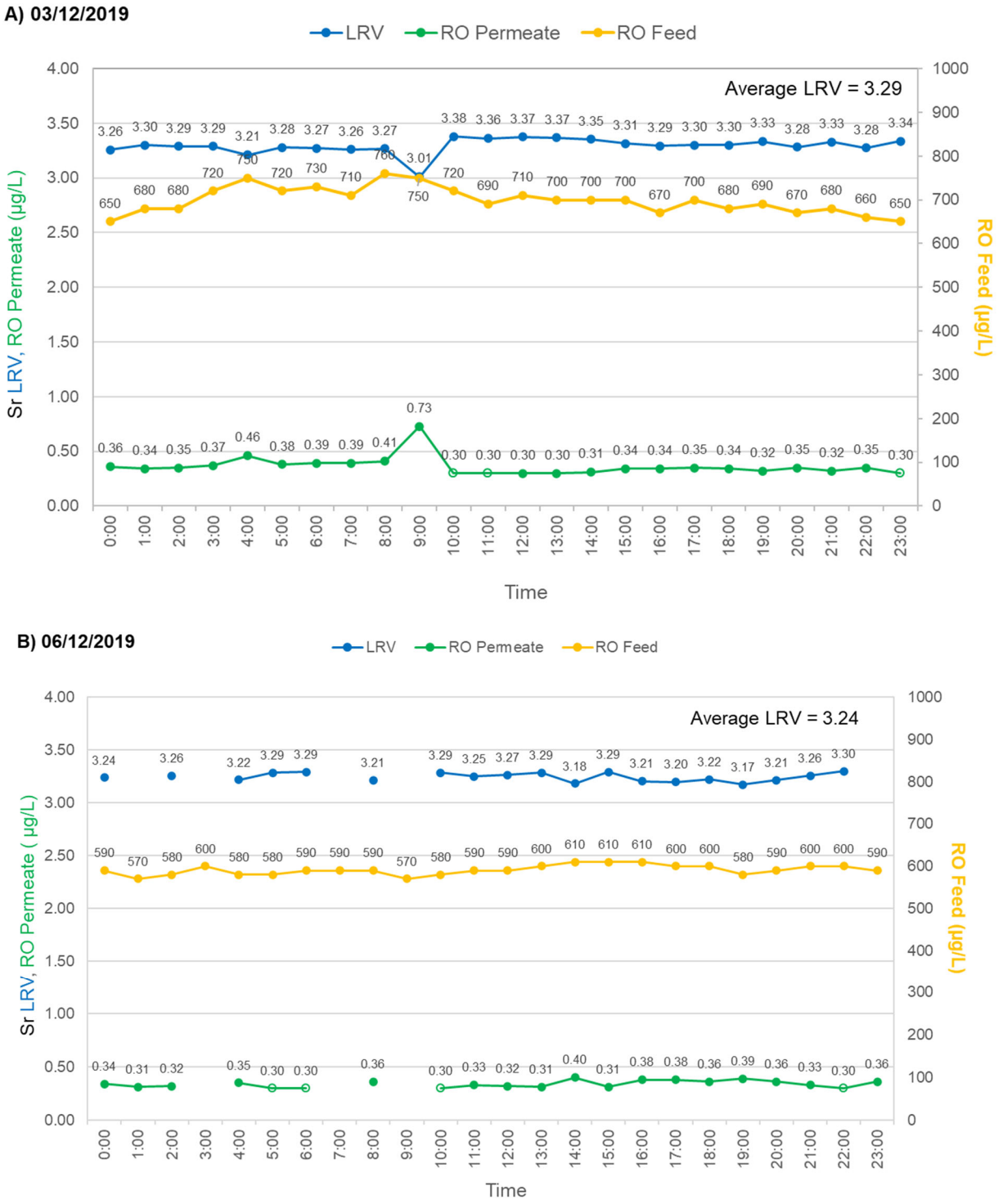
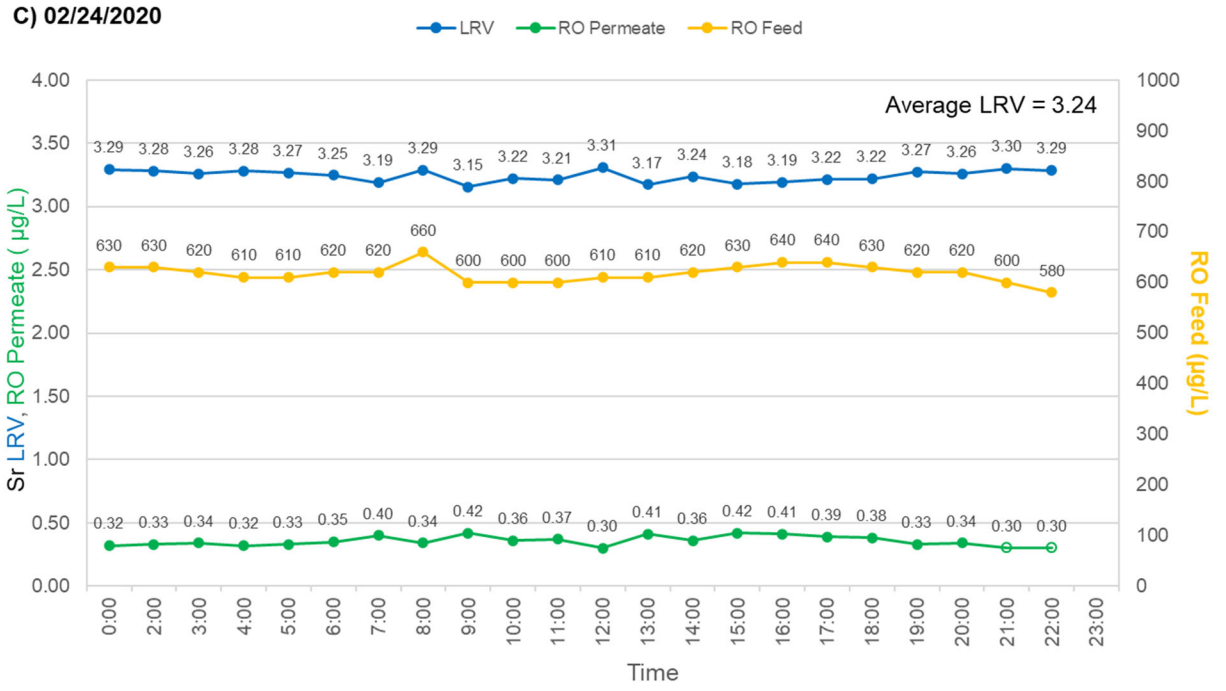


Figure 9-5. Strontium Rejection across a 5-MGD RO Unit.

Charts A through D represent strontium removal with Hydranautics ESPA2-LD RO membranes. Chart E* represents strontium removal with Dupont FilmTec BW30XFRLE, installed 10/31/2020. RO feed concentration of strontium (yellow) and permeate concentration (green) are also shown.

Source: Tackaert et al. 2021.

(continued)



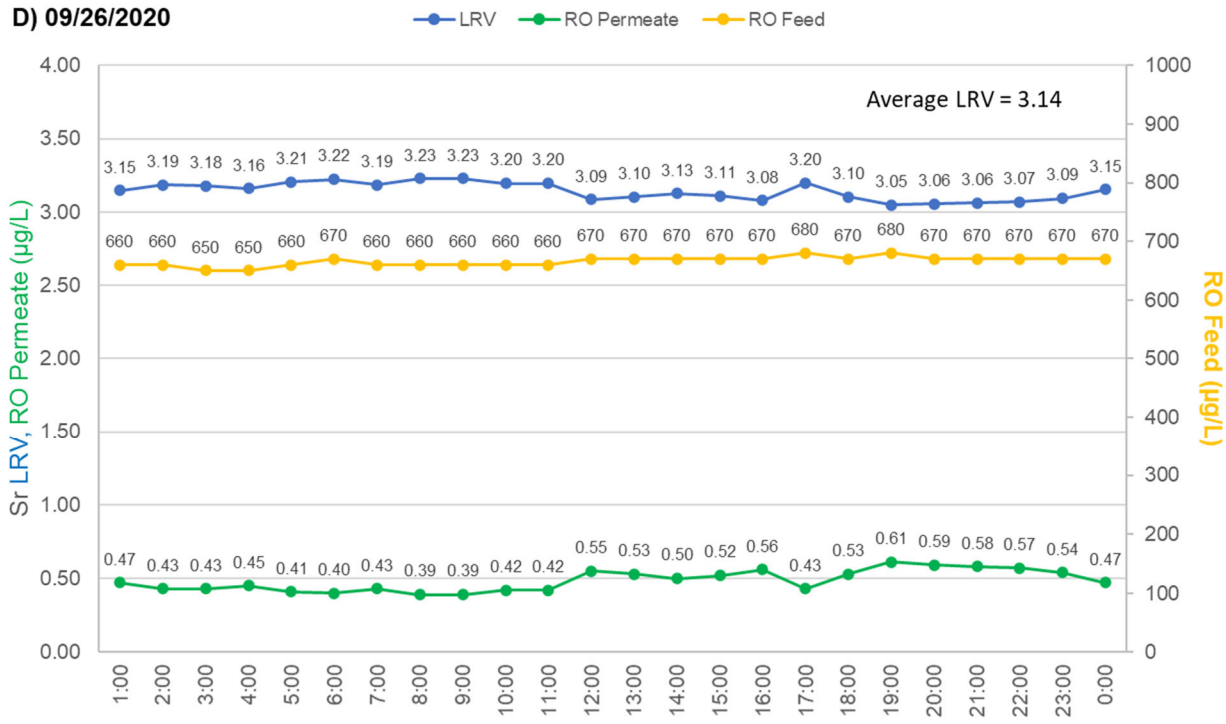


Figure 9-5. Continued.

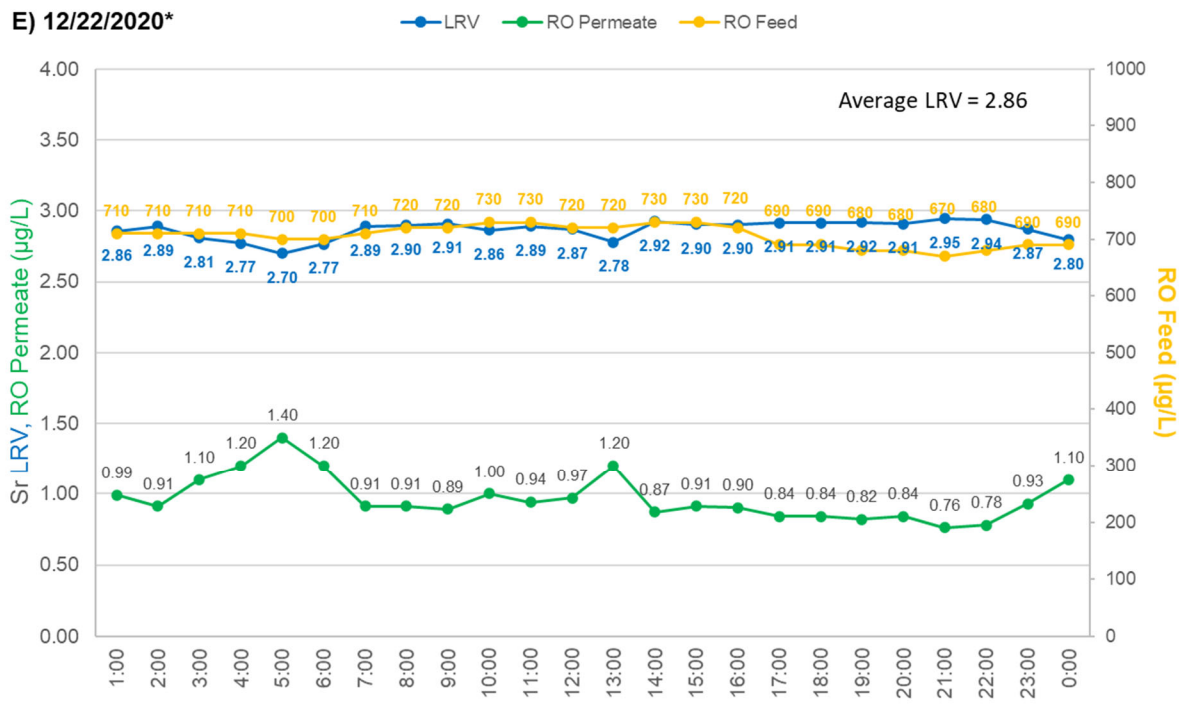


Figure 9-5. Continued.

Table 9-1. Grab Sample LRV Measurements for OCWD 5-MGD RO Unit after Installation of New Dupont FilmTec BW30XFRLE Membranes.

	LRV	
	Strontium	**Sulfate**
12/22/2020	2.86[a]	2.94[a]
1/21/2021	3.14	2.79
3/12/2021	3.08	3.03
4/14/2021	3.08	3.07
5/17/2021	NA	NA
7/13/2021	3.12	3.07

a – average LRV of 24-hr hourly sampling event
NA – not available
Source: Tackaert et al. 2021.

To capture LRV differences across the facility, additional strontium and sulfate grab samples were collected on full-scale 5-MGD RO units from the same plant featuring different brands and ages of membranes as well as on bulk permeate from all RO units (combined permeate from 21 RO units that proceeds as a blend to the UV/AOP treatment step). The LRVs for these additional samples are provided in Table 9-2 (strontium) and Table 9-3 (sulfate). They indicate that both the Hydranautics ESPA2-LD, Dupont BW30XFRLE and LG BW 400 ES membranes are capable of providing 3-logs of strontium rejection. All three membrane types are capable of providing ≥2.8-logs of sulfate rejection. The data shows there is some unit-to-unit variability for both strontium and sulfate rejection, but the bulk permeate LRV remains >3 for strontium and >2.8 for sulfate. On a bulk permeate basis, the LRVs for both sulfate and strontium slightly improved for December 2020 when some RO units had membranes replaced.

Table 9-2. Additional Strontium Sampling on OCWD AWPF RO Units.

Strontium Log Removal Values								
Membrane	**RO Unit**	**9/26/ 2020**	**12/22/ 2020**	**1/21/ 2021**	**3/12/ 2021**	**4/14/ 2021**	**5/17/ 2021**	**7/13/ 2021**
ESPA2-LD/ Dupont FilmTec BW30XFRLE[a]	B01	3.15[b]	2.89[c]	3.14	3.08	3.08	NA	3.12
ESPA2-LD	D03	3.22	3.40	1.94	3.30	3.40	3.40	3.41
DOW XFRLE[d]	G03	2.89	3.10	3.40	3.30	3.40	3.40	3.40
Dupont FilmTec BW30XFRLE	C01	NA	NA	NA	2.93	2.89	2.99	2.99
LG BW 400 ES	A01	NA	NA	NA	3.30	3.45	3.40	3.31
	Bulk Permeate[e]	3.15	3.10	3.15	3.33	3.33	3.34	3.27

a – 10/31/2020 ESPA2-LD membranes were replaced with DuPont FilmTec BW30XFRLE
b – ESPA2-LD membranes
c – DuPont FilmTec BW30XFRLE – 12/22/2020 to 07/13/2021
d – Older DuPont FilmTec BW30XFRLE membranes – Installed May 2015
e – Bulk permeate from all RO units at GWRS that are fitted with different membranes
NA – not available
Source: Adapted from Tackaert et al. 2021.

Table 9-3. Additional Sulfate Sampling on OCWD AWPF RO Units.

Sulfate Log Removal Values								
Membrane	**RO Unit**	**9/26/ 2020**	**12/22/ 2020**[c]	**1/21/ 2021**	**3/12/ 2021**	**4/14/ 2021**	**5/17/ 2021**	**7/13/ 2021**
ESPA2-LD/Dupont FilmTec BW30XFRLE	B01[a]	2.92[b]	2.96	2.79	2.82	2.92	NA	2.92
ESPA2-LD	D03	2.92	2.96	2.78	2.82	2.92	2.88	2.92
DOW XFRLE	G03	2.82	2.96	2.92	2.53	2.62	2.88	2.92
Dupont/FilmTec BW30XFRLE	C01	NA	NA	NA	2.82	2.89	2.88	2.92
LG BW 400 ES	A01	NA	NA	NA	2.82	2.92	2.88	2.92
	Bulk Permeate[d]	2.92	2.96	2.92	2.82	2.92	2.88	2.92

a – 10/31/2020 ESPA2-LD membranes were replaced with DuPont FilmTec BW30XFRLE
b – ESPA2-LD membranes
c – DuPont FilmTec BW30XFRLE – 12/20/2020 to 07/13/2021
d – Bulk permeate from all RO units at GWRS that are fitted with different membranes
NA – not available
Source: Tackaert et al. 2021.

9.4 Nanoparticles

The nanoparticle analysis did not deliver as expected based on prior research conducted by the research team for OCWD MF process feed/effluent (Rajagopalan et al. 2021), though that prior work was using a different nanoparticle analyzer no longer supported by that particular company. For the present study, data generated by the NTA software was not reproducible and

only background noise was recorded in the RO permeate and often in the RO feed. This could have been caused by either the instrument not being sensitive enough for the application or the concentration of nanoparticles was too low in the OCWD samples. During testing, the research team worked with Particle Metrix engineers to standardize the sampling method and data analysis but were not successful. Particle Metrix engineers are working on improvements and updates to their equipment and software which may improve nanoparticle detection for future applications.

9.5 Conclusions and Next Steps for OCWD

The goal of testing was to evaluate naturally occurring candidate surrogates that have potential to increase virus removal credits for the RO membrane treatment process, with a preference for surrogates that can be measured via online instrumentation. If a new surrogate is implemented, a facility can continue monitoring online TOC as supplemental organics removal monitoring and to serve as a back-up for virus removal credit in the event that for any reason the new surrogate(s) were not available. The findings from OCWD testing showed that free ATP, fluorescence Peak C, sulfate and strontium are four possible surrogates that can be used for this purpose which all demonstrated average LRVs that exceed current typical LRVs achieved by use of TOC or EC. Strontium, sulfate and free ATP are naturally occurring surrogates that showed the highest removal by the RO system with an average LRV of 3.29, 2.97 and 3.03, respectively, with strontium and sulfate determined from grab sampling and free ATP measured online. However, online strontium and sulfate analysis technologies (separate instruments) have recently become available and thus could be considered if preferable over grab samples. Online fluorescence Peak C was also noteworthy but more conservative with an average LRV of 2.70.

In the case of online free ATP, the minimum observed LRV was 2.60 such that it was always at least 0.5-log above the current TOC-based LRV credit for OCWD AWPF RO of approximately 2.0 LRV. Irrespective of the minimum daily value, it is expected that a plant could base credit on the average daily LRV (for free ATP or other online surrogates) as is currently permitted for OCWD for TOC-based virus LRV credit. For free ATP, the observed average daily LRV ranged 2.75 to 3.13, representing a minimum approximately 0.75-log increase over current use of TOC at approximately 2.0 LRV.

Online fluorescence features much less expensive instrumentation that is easier to calibrate and maintain compared to ATP (or online sulfate or strontium). The minimum observed LRV in this study was 2.27, while the average daily LRV ranged between 2.50 to 2.88, indicating that for OCWD this surrogate may not have much advantage over current use of TOC at approximately 2.0 LRV (i.e., approximately 0.5-log credit increase). On the other hand, OCWD leadership has noted that even a 0.5-log increase in credits for any unit process is meaningful and may be worth pursuing if available despite added cost or complexity. Further, other facilities may exhibit higher RO feed fluorescence leading to perhaps more potential for this surrogate, as with any surrogate.

With respect to naturally occurring ions, LRVs for sulfate and strontium were very stable and maintained in a narrow, steady range compared to the higher observed variability in LRV for

ATP and fluorescence. Strontium shows the greatest LRV for potential virus credits but would require a permanent program of frequent grab sampling or validation of an online analyzer. Sulfate is the next-highest LRV and would also require a permanent program of frequent grab sampling or validation of an online analyzer. Assuming online strontium or sulfate instrumentation performance is acceptable, the data from this study featuring high-frequency (hourly) strontium and sulfate grab samples can be considered as a simulation of the potential online data. The very stable concentrations (and LRVs) over the sampling period suggest that frequency of online sampling for strontium or sulfate could be minimized, i.e., daily or every few hours depending on regulatory requirements, which could reduce instrument maintenance and operating costs.

Based on this study, in the recent OCWD Title 22 Engineering Report (OCWD and DDB 2022) related to permitting the GWRS Final Expansion (under review by DDW at the time of this report), OCWD proposed to use on-line ATP, sulfate or strontium as the primary surrogates in a tiered approach for performance indicators for virus LRV credit by the RO process for the GWRS and GWRS Final Expansion. The implementation of the monitoring programs is still being finalized at the time of this report, but either free ATP or sulfate or strontium analyzers are proposed to be installed on a permanent basis. On-line analyzers installed on the common headers (bulk) of the RO feed and RO permeate streams are proposed to measure free ATP, sulfate or strontium concentrations continuously and track RO performance. However, it is expected that DDW may require some degree of RO unit-specific monitoring (i.e., permeates from different parallel 5-MGD RO units), as opposed to only on the blended permeate (bulk permeate).

APPENDIX A

Covariance Tool

Estimating LRV from Correlated Influent-Effluent Data
(Example Trickling Filter Data from Plant 1)

			Ranked Data From TF at P1			
No.	F[No]	Probits	Influent	Effluent	log(Infl)	log(Effl)
1	2.6%	-1.95	55	9	1.74	0.94
2	6.7%	-1.50	128	11	2.11	1.06
3	10.8%	-1.24	132	12	2.12	1.09
4	14.9%	-1.04	250	15	2.40	1.17
5	19.1%	-0.88	361	23	2.56	1.36
6	23.2%	-0.73	388	52	2.59	1.71
7	27.3%	-0.60	775	66	2.89	1.82
8	31.4%	-0.48	875	88	2.94	1.94
9	35.6%	-0.37	1,060	88	3.03	1.94
10	39.7%	-0.26	1,083	91	3.03	1.96
11	43.8%	-0.16	1,222	93	3.09	1.97
12	47.9%	-0.05	1,228	186	3.09	2.27
13	52.1%	0.05	1,292	204	3.11	2.31
14	56.2%	0.16	1,303	226	3.11	2.35
15	60.3%	0.26	1,519	240	3.18	2.38
16	64.4%	0.37	1,742	250	3.24	2.40
17	68.6%	0.48	1,841	254	3.26	2.40
18	72.7%	0.60	2,727	290	3.44	2.46
19	76.8%	0.73	5,141	309	3.71	2.49
20	80.9%	0.88	5,587	480	3.75	2.68
21	85.1%	1.04	6,666	659	3.82	2.82
22	89.2%	1.24	10,438	924	4.02	2.97
23	93.3%	1.50	12,372	1,623	4.09	3.21
24	97.4%	1.95	45,298	2,016	4.66	3.30
	5%	-1.64				
	50%	0.00				
	95%	1.64				
	99.99%	3.719				
	0.01%	-3.72				

STEP 1: Data for influent and effluent are pasted in to columns D and E

NOTE: the number of entries in each column must be the same

STEP 2: Use the Excel SORT function to rank the influent and effluent data columns separately from smallest to largest (i.e. the data in each column are ranked)

STEP 3: Calculate Covariance using the Excel COVARIANCE.S function

COVARIANCE	0.439

If Covariance is positive, Log Effl increases as Log Infl increases

If covariance is negative, Log Effl decreases as Log Infl increases

STEPs 4 & 5: Study the correlation between Log Infl and Log Eff

STEP 4: Estimate statistical significance of correlation

Examining the statistical significance of the correlation of the ranked data:

Pearson's correlaton coefficient, r*	0.972
Sample size, N	24
Calculation of Pearson's P-value**	2.66E-15
p =	<0.001

Correlation between log-infl and log-effl is significant

Notes on use of P-value

*the Pearson product moment correlation coefficient, r, a dimensionless index the value of "r" that ranges from -1.0 to 1.0 inclusive and reflects the extent of a linear relationship between two data sets.

**The P-value is the probability that you would have found the current result if the correlation coefficient were in fact zero (null hypothesis).

The conventional criterion for acceptance is a probability of 5% or 0.05

- If p < 0.05, There the relationship between the Log of the influent concentration and the Log of the effluent concentration is considered significant.
- If p ≥ 0.05 the observed relationship is not considered significant

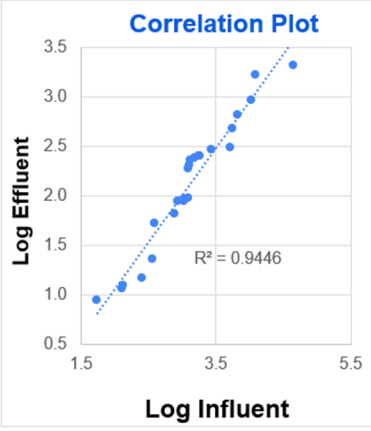
According to convention, the actual vallue of p is only shown if

it is between 0.001 and 0.05

values above 0.05 are shown as "p > 0.05"

Values below 0.001 are shown as "p < 0.001"

STEP 5: Study the correlation plot:



STEP 6: Calculate the 5th %-tile LRV using covariance

a) Calculate Average log-values and LRV

	log-Inf	log-Eff	LRV
avg =	3.124	2.125	0.999

b) Calculate variance of the log-values

	log-Inf	log-Eff
Var =	0.463	0.441

c) Establish %-tile of interest and find Z-score (Probits)

for X% Probits = Z-score is found with Excel Function NORM.S.INV(x%)

x%	Z-score
5%	-1.645

d) Estimate variance of [log-inf - log-eff]

Var-Δ = Var-log-inf + Var-log-eff - 2*Covariance

Var-Δ =	0.026

e) Estimate Std Dev of [log-inf - log-eff]

StdDev-Δ =	0.160

f) Estimate LRV at x%-tile

LRV at x%-tile = Average LRV + Z-score*StdDev-Δ

LRV at x%-tile	**0.736**

References

Adham, S S., Trussell, R.S., Gigliardo, P.F., and Trussell, R.R. 1997. "Rejection of MS-2 Virus by RO Membranes." *Journal AWWA,* 90.9: 130-135.

Antony, A., Blackbeard, J., Angles, M., and Leslie, G. 2014. "Non-microbial Indicators for Monitoring Virus Removal by Ultrafiltration Membranes." *Journal of Membrane Science*, 454: 193-199.

Bartolo, M. 2018. Achieving Pathogen Credit through Wastewater Treatment. Presented by Mitchel Bartolo, P.E., Trussell Technologies for 33rd Annual WateReuse Symposium. September 2018.

Bernados, B. 2018. "Reverse Osmosis for Direct Potable Reuse in California." *Journal of AWWA*, 110.1: E28-E36.

City of San Diego. 2019. *Final Draft Title 22 Engineering Report – North City Pure Water Project*. Public Utilities Department. https://www.sandiego.gov/sites/default/files/north_city_pure_water_project_final_draft_title_22_engineering_report.pdf.

Debroux, J., Plumlee, M.H., and Trussell, S. 2021. *Defining Potential Chemical Peaks and Management Options*. Project 4991. Denver, CO: The Water Research Foundation.

DeCarolis, J., Adham, S., Kumar, M., Pearce, B., and Wasserman, L. 2005. Integrity and Performance Evaluation of New Generation Desalting Membranes During Municipal Wastewater Reclamation. WEFTEC. Washington, DC: Water Environment Federation.

EPA (U.S. Environmental Protection Agency). 2001. *Method 1602: Male-Specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure*. EPA.

EPA (U.S. Environmental Protection Agency). 2010. *Method 1615: Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR. Version 1.3*. Revised September 2014. www.epa.gov/ord.

EPA (U.S. Environmental Protection Agency). 2005. *Membrane Filtration Guidance Manual*. Cincinnati, OH: EPA.

Gerrity, D., Gamage, S., Holady, J.C., Mawhinney, D.B., Quinones, O., Trenholm, R.A., and Snyder, S.A. 2011. "Pilot-Scale Evaluation of Ozone and Biological Activated Carbon for Trace Organic Contaminant Mitigation and Disinfection." *Water Research,* 45.5: 2155-2165.

Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M., and Khan, S.J. 2009. "Fluorescence as a Potential Monitoring Tool for Recycled Water Systems: A Review." *Water Research,* 43: 863-881.

Jacangelo, J.G., Subramani, A., Cran, M., Gray, S., Knoell, T., Bashai, J., and Schwab, K. 2015. Microbial Seeding Studies for Demonstration of Log Removal in DPR: A Review of Methodology

and Field Applications. 30th Annual Water Reuse Symposium. Seattle, WA, 2015. September 13- 16.

Kimura, K., Amy, G., Drewes, J.E., Heberer, T., Kim, T.U., and Watanabe, Y. 2003. "Rejection of Organic Micropollutants (Disinfection By-products, Endocrine Disrupting Compounds, and Pharmaceutically Active Compounds) by NF/RO Membranes." *Journal of Mem. Sci.,* 227: 113-221.

Kruithof, J.C., Kamp, P.C., Folmer, H.C., Nederlof, M.M., and van Hoof, S.C.J.M. 2001. "Development of a Membrane Integrity Monitoring Strategy for the UF/RO Heemskerk Drinking Water Treatment Plant." *Water Sci. Tech.: Water Supply,* 1.5: 261-271.

MacDonald, B.C., Lvin, S.J., and Patterson, H. 1997. "Correction of Fluorescence Inner Filter Effects and the Partitioning of Pyrene to Dissolved Organic Carbon." *Analytical Chimica Acta,* 338.1-2: 155-162.

Maki, J.S., LaCroix, S.J., Hopkins, B.S., and Stanley, J.T. 1986. "Recovery and Diversity of Heterotrophic Bacteria from Chlorinated Drinking Waters." *Appl. Environ. Microbiol.,* 5.5: 1047-1055.

McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, P.T., Kulbe, T., and Andersen, D.T. 2001. "Spectrofluorometric Characterization of Dissolved Organic Matter for Indication of Precursor Organic Material and Aromaticity." *Limnology and Oceanography,* 46.1: 38-48.

NWRI (National Water Research Institute). 2018. *Subcommittee Comments on the Draft Enteric Virus Log Removal Study Test Plan, Orange County Sanitation District Plants No. 1 and 2*. Prepared by an NWRI subcommittee for the Orange County Water District, Groundwater Replenishment System (GWRS).

NWRI (National Water Research Institute). 2019. *Subcommittee Report on Statistics and Pathogen Monitoring*. Draft. Prepared by an NWRI IAP subcommittee for the City of San Diego, Public Utilities Department.

NWRI (National Water Research Institute). 2020. *Review of the Orange County Water District Groundwater Replenishment System*. Prepared by NWRI Independent Advisory Panel to review the Groundwater Replenishment System for the Orange County Water District.

OC San (Orange County Sanitation District). 2015. *Capital Improvement Program Fiscal Year 2014/15 Update*. Orange County Sanitation District.

OCWD (Orange County Water District). 2015. *Groundwater Replenishment Reuse Project Compliance Report, Orange County Water District, Groundwater Replenishment System.* Submitted to State Water Resources Control Board, Division of Drinking Water.

OCWD (Orange County Water District). 2019. *Final Study Test Plan to Determine Enteric Virus Log Reduction Values (LRVs) from Orange County Sanitation District Plants No. 1 and No. 2*. OCWD.

OCWD and DDB (Orange County Water District & DDB Engineering, Inc.). 2022. *Title 22 Engineering Report Update for the Groundwater Replenishment System and Final Expansion.* OCWD and DDB.

Olivieri, A., Crook, J., Anderson, M., Bull, R., Drewes, J., Haas, C., Jakubowski, W., McCarty, P., Nelson, K., Rose, J., Sedlak, D., and Wade, T. 2016. *Expert Panel Final Report: Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse.* Prepared for California State Water Resources Control Board Division of Drinking Water and Submitted by National Water Research Institute, Sacramento, California.

Ozaki, H., and Li, H. 2002. "Rejection of Organic Compounds by Ultra-Low Pressure Reverse Osmosis Membrane." *Water Res.,* 36:123-130.

Pecson, B., Darby, E., Di Giovanni, G., Leddy, M., Nelson, K., Rock, C., Slifko, T., Jakubowski, W., and Olivieri, A. 2021. *Pathogen Monitoring in Untreated Wastewater*. Project 4989. Denver, CO: The Water Research Foundation.

Rajagopalan, G., Safaee, H., and Holloway, R. 2021. *Improving Membrane Treatment Energy Efficiency through Monitoring and Removal of Colloidal Particles Foulants*. California Energy Commission.

Schmidt, P.J., Anderson, B.W., and Emelko, B.M. 2020. "Describing Water Treatment Process Performance: Why Average Log-Reduction Can Be a Misleading Statistic." *Water Research,* 176: 115702.

Schwartzman, A. 2018. *Pathogen Monitoring Study at the North City Water Reclamation Plant: Statistics Review and Analysis Report*. Prepared for the City of San Diego Public Utilities Department.

Siebel, E., Wang, Y., Egli, T., and Hammes, F. 2008. "Correlations between Total Cell Concentration, Total Adenosine Triphosphate Concentration and Heterotrophic Plate Counts during Microbial Monitoring of Drinking Water." *Drinking Water Engineering and Science,* 1.1: 1-6.

Singh, S., Henderson, R.K., Baker, A., Stuetz, R.M., and Khan, S.J. 2012. "Characterisation of Reverse Osmosis Permeates from Municipal Recycled Water Systems Using Fluorescence Spectroscopy: Implications for Integrity Monitoring." *Journal of Membrane Science,* 412-422: 180-189.

SM (Standard Methods for the Examination of Water and Wastewater). 2018. *Standard Method 9222: Membrane Filter Technique for Members of the Coliform Group*. DOI: 10.2105/SMWW.2882.193.

Stanford, B.D., Pisarenko, A.N., Holbrook, D.R., and Snyder, S.A. 2011. "Preozonation Effects on the Reduction of Reverse Osmosis Membrane Fouling in Water Reuse." *Ozone: Science and Engineering*, 33.5: 379-388.

SWRCP DDW (California State Water Resources Control Board, Division of Drinking Water). 2017. “Orange County Water District, Groundwater Replenishment System (GWRS), System No. 3090001, Project No. 745, Comments on Section 60320.200 Groundwater Recharge Report.” Letter dated January 27, 2017.

SWRCP DDW (California State Water Resources Control Board, Division of Drinking Water). 2019. *Approval Letter for the Orange County Water District Groundwater Replenishment System’s Enteric Virus Log Reduction Values Study Test Plan (3090001-756)*. California State Water Resources Control Board Division of Drinking Water.

SWRCP DDW (California State Water Resources Control Board, Division of Drinking Water). 2021. *A Proposed Framework of Regulating Direct Potable Reuse in California Addendum*. Prepared by State Water Board’s Division of Drinking Water.

Tackaert, R., Pisarenko, A., Idica, E., Trussell, S.R., Safarik, J., and Plumlee, M.H. 2021. *Novel Online Surrogates to Monitor Reverse Osmosis Performance in Reuse Applications*. Draft. USBR.

Tchobanoglous, G., and Hardy, K.M. 2017. *Panel Findings and Recommendations Based on a Meeting Held October 9-10, 2017, to Review the City of San Diego’s Pure Water Program*. Prepared by an NWRI IAP for the City of San Diego, Public Utilities Department.

Tchobanoglous, G., Kenny, J., and Leverenz, H. 2021. “Rationale for Constant Flow to Optimize Wastewater Treatment and Advanced Water Treatment Performance for Potable Reuse Applications.” *Water Environ Res.* doi: 10.1002/wer.1531.

Trussell Technologies. 2017a. *Pathogen Monitoring Study at the North City Water Reclamation Plant.* Prepared for Public Utilities Department City of San Diego, California.

Trussell Technologies. 2017b. Monitoring Pathogen Concentrations through the City of Oceanside’s San Luis Rey Wastewater Treatment Plant. 11th International Water Association International Conference on Water Reclamation and Reuse, July 23-27, 2017.

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