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Evaluation of Tier 3 Validation Protocol for Membrane Bioreactors to Achieve Higher Pathogen Credit for Potable Reuse

CALIFORNIA Water Boards
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Evaluation of Tier 3 Validation Protocol for Membrane Bioreactors to Achieve Higher Pathogen Credit for Potable Reuse

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Abstract and Benefits

Abstract:

Research has demonstrated the ability of membrane bioreactor (MBR) systems to provide significant virus, protozoa, and bacteria removal. However, a gap exists in the regulatory application of pathogen log removal value (LRV) credits for MBR systems within potable reuse systems. Without a clear regulatory pathway to approve higher LRVs for MBRs, potable reuse agencies are faced with installing additional treatment and/or costly monitoring systems to improve pathogen credits for other processes. The Australian WaterVal program described three Tiers of validation to obtain LRV credits for viruses, bacteria, and protozoa. This project evaluated the Tier 3 WaterVal MBR validation protocol for test cases in the United States, identified modifications needed to adapt the Tier 3 protocol for the United States, and developed implementation recommendations for a Tier 3 style regulatory approach for increased operational flexibility in reuse facilities.

Benefits:

- Review and summary of the various monitoring strategies for MBR that are available to date and their perceived relationship with pathogen removal mechanisms.
- A comparison of existing Australian MBR Validation Guidance was made with recent WRF projects and relevant US Water Reuse Paradigms, providing perspective on the direction of the industry.
- Membrane Suppliers and Utility representatives were surveyed on different MBR validation Tiers to understand the perceived value of Tier 1, Tier 2, and Tier 3 MBR validation.
- New analysis of a prior WaterVal case study data demonstrates the potential of turbidity and microbiological LRV relationships.
- A general approach to Tier 3 validation is provided, informed by a technical advisory workshop and available case studies.

Keywords: membrane bioreactor, MBR, validation, Tier 3, pathogen removal, log reduction value, LRV, surrogates

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Acronyms and Abbreviations

APC	Advanced Purification Center
µm	Micrometers
AOP	Advanced oxidation process
AWPF	Advanced Water Purification Facility
AWTF	Advanced Water Treatment Facility
BAC	Biologically Active Carbon
CAS	Conventional activated sludge
CCL	Critical control limit
CCP	Critical control point
CCR	California Code of Regulations
CFU/L	Colony forming units per liter
COD	Chemical oxygen demand
DDW	California Division of Drinking Water
DIT	Direct integrity test
DPR	Direct potable reuse
gfd	Gallons per square foot per day
g/L	Grams per liter
HACCP	Hazard and Critical Control Point
HRT	Hydraulic retention time
IEP	Isoelectric point
IPR	Indirect potable reuse
L	Liter
LRV	Log reduction value
MBR	Membrane bioreactor
MC	Maintenance clean
MF	Microfiltration
MFGM	Membrane Filtration Guidance Manual
mg/L	Milligrams per liter
mgd	Million gallons per day
MiQE	Minimum Information for Publication of Quantitative Real-Time PCR Experiments
MLSS	Mixed liquor suspended solids
MPN/L	Most probable number per liter
MWDSC	Metropolitan Water District of Southern California
NaCl	Sodium chloride
nm	Nanometers
NTU	Nephelometric turbidity unit
O&M	Operation and maintenance
O3	Ozone
PDR	Pressure decay rate
PDT	Pressure decay test
PMMoV	Pepper Mild Mottle Virus

Q	Permeate flow
QA	Quality assurance
QC	Quality control
RAS	Recycled activated sludge
RC	Recovery clean
RMSE	Root mean squared error
RO	Reverse osmosis
SCVWD	Santa Clara Valley Water District
SRT	Solids retention time
SWRCB	State (of California) Water Resources Control Board
TAC	Technical advisory committee
TMP	Transmembrane pressure
TOC	Total organic carbon
TSS	Total suspended solids
UF	Ultrafiltration
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVT	UV transmittance
VCF	Volumetric concentration factor
WAS	Waste activated sludge
WRF	Water Research Foundation
WW	Wastewater

Executive Summary

Membrane bioreactors (MBRs) represent a potential cost and footprint saving while providing high-quality effluent compared to conventional activated sludge systems. As a result, some potable water reuse projects across the United States and globally have decided to have MBRs as part of the potable reuse treatment train.

Reduction of pathogens in potable reuse is a primary focus, due to the potential for acute health impacts. Microbiological analysis turnaround times are at least 24 hours for indicator microorganisms and may be weeks or months for pathogenic protozoa and viruses. Such response times are not sufficient to verify water quality, considering distribution times of water in potable reuse schemes. Hazard and critical control point (CCP) frameworks are increasingly incorporated to ensure that appropriate water quality is verified in real time during potable reuse. Successful application of CCPs requires installation and demonstration of online monitored parameters to indicate the treatment effectiveness of significant pathogen removal barriers (or mechanisms). To understand the complexity of CCPs for MBR, one has to first look at the relative simplicity of CCPs for measurement of pathogen log reduction values (LRVs) by ultraviolet light (UV) disinfection, which incorporates real time monitoring of water quality, flow, UV intensity, UV dose, and use of UV reactors validated in accordance with industry protocols. MBRs remove pathogens via a number of mechanisms including size exclusion by the membrane, enhanced size exclusion by the dynamic fouling layer, biological predation within the activated sludge, and adsorption to sludge flocs and subsequent wasting. The effectiveness of each of these mechanisms is pathogen specific. The complexity of pathogen removal mechanisms in MBRs has so far prevented development of a rigorous CCP based method to validate treatment performance.

Earlier research in Australia through the WaterVal program examined a 3-tiered validation framework as a means to provide a pragmatic approach to the complexity of validating multiple mechanisms of pathogen removal in MBRs. Briefly, the tiered approach included the following levels:

- Tier 1 - Accredits performance based on analysis of existing LRV data for MBRs but uses statistically conservative 5th percentile performance values
- Tier 2 - Requires demonstration of MBR product-specific performance through extensive testing, followed by an ongoing monitoring protocol to demonstrate facility LRVs
- Tier 3 - Requires implementation of a CCP based method to validate treatment performance ideally in real time

The Water Research Foundation's project 4997 (Salveson et al., 2021) re-examined and developed Tier 1 and Tier 2 concepts for MBRs in potable reuse applications in the United States, resulting in broad and conservative Tier 1 virus and protozoa LRV credit and a step-by-step approach for higher Tier 2 LRV credits. The goals of this Tier 3 project were to:

- Evaluate the proposed WaterVal Tier 3 approach, considering how to correlate pathogen LRV and a monitoring system result
- Survey utilities and suppliers to best understand the relative value of a Tier 3 system compared to Tier 1 and Tier 2 systems
- Develop recommendations for how a Tier 3 protocol should be designed
- Develop a U.S.-based MBR Validation Protocol Outline for Tier 3.

In Chapter 2, the mechanisms of pathogen removal and anticipated relationships with controlled (including design or operational) and monitoring parameters were reviewed and discussed. In general, there are no single monitoring techniques that can be significantly correlated to pathogen removal. However, a number of techniques can be used to infer the effectiveness of certain mechanisms. For example, elevated turbidity in MBR filtrate or a higher rate of pressure loss in a pressure decay test (PDT) are likely due to membrane integrity failure and a potential breakdown of size exclusion mechanisms. Based on prior research, a majority of pathogen removal can be achieved if the membrane barrier is intact. To that end, Tier 3 approaches were distinguished based on:

- Approach 1 - Monitoring verification of the membrane barrier with either modelling or empirical study to account for bioreactor removal
- Approach 2 - Separate monitoring techniques to monitor and relate to bioreactor and membrane removal efficacy
- Approach 3 - Development of a single surrogate that can be correlated with overall removal

Approach 1 was initially proposed for turbidity as part of WaterVal. The initial model development is described in Chapter 2 and a recent case study of a full-scale facility was used to evaluate the site-specific ability of the turbidity Approach 1. The small amount of full-scale case study results suggested that:

- The empirical use of 5th percentile bioreactor LRVs in conjunction with turbidity validation to determine LRV across the membrane would result in conservative LRVs.
- Given the logarithmic relationship between turbidity and membrane LRV, very large changes in turbidity were required to significantly reduce MBR LRV.
- An optimization exercise suggested that the adopted LRVs for the bioreactor may be underestimating the actual contribution to overall LRV at the site tested and is thus conservative.

In Chapter 3, representative membrane suppliers and partner utilities that operate MBR facilities were surveyed about the tiered framework. The operational data collected from suppliers highlighted that the operational envelope of results from WaterVal was too restrictive to cover the potential operational range of MBRs. More importantly, both utilities and suppliers considered that the Salveson et al., 2021 Tier 1 protozoa and virus LRVs of 2.5 and 1.0, respectively, were conservative but sufficient to allow risk-free substitution of conventional activated sludge with MBRs in indirect potable reuse schemes. Further, the Tier 2 protocol provided as part of Salveson et al., 2021 was an avenue for specific suppliers to demonstrate higher credit, which could then be used to control risk as part of direct potable reuse schemes

by providing excess LRV as a safety margin. Both suppliers and utilities acknowledged that a Tier 3 framework would provide the ability to rapidly respond to potential treatment upsets and, in doing so, provide more efficient risk reduction. However, the following concerns were raised with respect to a Tier 3 framework, namely:

- There is no clearly described Tier 3 validation guidance nor are their case studies of regulatory approvals for such efforts (such as Salveson et al., 2021 for Tier 1 and Tier 2). Without such guidance, Tier 3 is unlikely to be attempted.
- Tier 3 is anticipated to require a shift to greater instrument maintenance, verification and calibration costs. Any Tier 3 strategy must be based on robust instrumentation.
- There did not appear to be an increase in the amount of LRV possible with Tier 3 over Tier 2 (for which guidance and protocols are established).

A workshop was held with the entire project team as well as technical advisors. During the workshop, focus questions were addressed to help scope the required elements of a Tier 3 protocol. In general, workshop participants agreed that the following elements were important to consider when monitoring MBRs to validate pathogen LRV:

- Current monitoring techniques with a maximum resolution of daily, such as pressure decay testing or indicator microorganism sampling, could be considered, provided there was a real-time indicator that could trigger corrective action in a timely fashion in the event of gross process failure. The only identified online monitoring trigger at this time is turbidity.
- Pathogen data must be corrected for recovery. With the use of non-standard DNA-based analysis techniques, additional quality assurance is established in literature and should be followed to avoid erroneous results.
- There is no evidence to suggest that the sampling requirement for Tier 3 should be less than that established for Tier 2 (i.e., at least 20 samples so that a meaningful 5th percentile limit can be set). For Tier 3, this may mean that multiple sets of 20 samples under different monitoring limits may need to be gathered to demonstrate sufficient sensitivity to the 5th percentile, which results in more sampling required for Tier 3 compared to Tier 2.
- Depending on project-specific needs, it should be possible to conduct Tier 3 validation for a subset of pathogens (i.e., protozoa only or virus only). This was considered necessary as:
    - There may be reasons why a measurement technique correlates with protozoa and not viruses.
    - There may only be a project need to validate for protozoa removal as a satisfactory level of virus removal may already be present due to other validated unit operations.

Based on the experience of the project team, the data collected, and the workshop, a validation protocol outline was proposed in Chapter 4. Even at this time, specific guidance cannot be presented due to the scarcity of similar Tier 3 activities that have measured pathogenic microorganisms, and thus the guidance can only be expanded and detailed based upon future fact finding, either through more Tier 2 efforts or through exploratory Tier 3 efforts. Although Tier 3 was initially designed to be specific to a monitoring technique, and not membrane product specific, with very few sources of pathogen data correlated with surrogates and indicator microorganisms, it is recommended that Tier 3 validation be conducted at least for

each pairing of membrane product, monitoring technique, and facility design parameters (if they vary significantly). To that end, Tier 3 validation with currently available technologies may not significantly deviate from the guidance already available for Tier 2 validation as part of Salveson et al., 2021. However, when sufficient evidence is gathered to demonstrate that a monitoring technique can relate to MBR LRV, independent of membrane supplier, it is recommended that supplier specific verification requirements are waived.

Related WRF Research

- Membrane Bioreactor Validation Protocols for Water Reuse (4997)
- New Techniques, Tools, and Validation Protocols for Achieving Log Removal Credit Across NF and RO Membranes (4958)
- Use of DNA Nanostructure as Viral Surrogates in Potable Reuse Applications (5104)

CHAPTER 1

Introduction

Potable water reuse projects, in some instances, utilize membrane bioreactors (MBR) as part of the potable reuse treatment train. MBRs represent a potential cost and footprint saving while providing high-quality effluent.

Pathogen removal and the determination of pathogen log reduction credits, also called LRV, by MBR has been studied in Australia through the WaterVal program (WaterSecure, 2017) and in California by engineers and manufacturers, among many other locations. WaterSecure (2017) established the 3-Tiered program (described below) and the Water Research Foundation (Salveson et al., 2021) re-examined and developed Tier 1 and Tier 2 concepts for MBRs in potable reuse applications in the United States. The Tier 3 effort within the United States is defined here within this report.

1.1 Overview of Prior Validation Efforts

This section briefly summarizes the tiered approach to MBR validation proposed in previous work. The research program behind the WaterVal framework (Branch and Le-Clech, 2015) and the final validation protocol (WaterSecure, 2017) as well as the revised and updated recommendations for Tier 1 and Tier 2 validation in the US (Salveson et al., 2021) should be consulted for detailed information. The three Tiers are similar for both the Australian and US efforts.

In Tier 1 validation, MBRs are granted default LRV credits conditional on operating within a specific range of effluent water quality (e.g., turbidity). In both Australian and US validation guidelines, conservative LRVs were assigned a value corresponding to the 5th percentile from LRV data sets collected during WaterVal and later US validation studies (Branch and Le-Clech, 2015; SCVWD, 2017). The LRVs calculated in WaterVal, and later US validation studies considered slightly different input data sets, with more data available for Salveson et al., 2021. When the 5th percentile LRVs were calculated, the following values were proposed for default credits:

- WaterVal (Water Secure, 2017) set the minimum values of:
  - Protozoa - 2 LRV.
  - Bacteria - 4 LRV.
  - Virus - 1.5 LRV.
- Salveson et al., 2021 set the minimum values of:
  - Protozoa - 2.5 LRV.
  - Virus - 1.0 LRV.

The data set for Salveson et al., 2021 included all of the WaterVal source data, in addition to the Santa Clara Valley Water District (SCVWD) study, which analyzed a wider array of organisms. The protozoa LRV from WaterVal was based on *C. perfringens*, whereas, the results

from the SCVWD study shows that *Giardia* LRV was much higher. Lower removal of somatic coliphage was noted from the SCVWD study. Accordingly, a decrease in the LRV from 1.5 to 1.0 for virus was adopted and a marginal increase in protozoa LRV from 2.0 - 2.5 when comparing Salveson et al., 2021 values to WaterVal.

Water Secure (2017) established an "operational envelope" for LRV credits to be valid, along with meeting turbidity criteria. The operational envelope corresponded to the range of operating conditions, under which the LRV data used for calculation of Tier 1 LRV credits was collected.

Salveson et al., 2021 intentionally **did not** specify an operational envelope, but required strict compliance with State of California turbidity criteria for membrane filtrate/filtered wastewater (§60301.320 b), [SWRCB, 2018]); which is:

- 0.2 Nephelometric turbidity unit (NTU) 95% of the time; and
- 0.5 NTU not to exceed.

In addition, implementation of a secondary monitoring technique, such as pressure decay testing or filtrate total coliform monitoring, to confirm membrane integrity was proposed on a voluntary basis.

Default bacteria LRVs were not investigated as part of Salveson et al., 2021 as the general assumption was that if virus and protozoa were effectively treated, bacteria would be as well, which is supported by the Tier 1 LRV of 4 for bacteria, relative to 2.0 for protozoa determined in WaterVal (WaterSecure, 2017). This same assumption has been made in key prior studies concerning pathogenic microorganism control in water reuse (Trussell et al., 2013).

In Salveson et al., 2021, Tier 1 LRVs are membrane supplier agnostic, applying regardless of supplier, provided the membranes have pore size less than 0.4 micrometers (µm). In addition, MBR suppliers are not required to perform preliminary challenge testing to demonstrate LRV of their system.

Tier 2 validation requires that MBRs undergo product and site-specific challenge testing to demonstrate an ability to reliably achieve higher LRV values than proposed for Tier 1.

Ongoing work suggests that virus LRV will be >2.0 and protozoa LRV will be >3.5 (Fontaine and Morris, 2020). Even when large sample volumes (c. 1000 liters [L]) of filtrate are analyzed, pathogens are often not detected post MBR treatment. As such, results are expressed as >LRVs.

Tier 2 testing is extensive, including pathogens, indicator microorganisms, membrane integrity verification and monitoring changes in performance over time.

For the Salveson et al., 2021 Tier 2 protocol, *Cryptosporidium* is the target protozoa and *Clostridium perfringens* is the proposed indicator microorganism. Culturable enteroviruses are the target virus and both male-specific and somatic coliphages are the proposed indicator microorganism.

The Tier 2 protocol proposed in Salveson et al., 2021 suggests sampling over three phases:

- Pre-commissioning - Testing to be performed on a particular membrane product at pilot or full scale:
  - The intent is to prove the capability of the product.
  - Minimum 24 samples from feed and filtrate testing indicators defined above and monitoring surrogates
  - Samples must be taken over a minimum time period of 3 months.
- Commissioning - Testing to be performed on a specific full-scale site to be validated.
  - The intent is to set site specific baseline LRVs,
  - Account for scale up from pilot challenge test data,
  - Confirm appropriate surrogate limits proposed in pre-commissioning testing,
  - Sampling twice per month for the first 12 months of operation.
  - If the new proposed site is smaller, or equivalent to that tested during pre-commissioning, then only indicators need to be analyzed. If not, then both indicators and target pathogens need to be analyzed.
- On-going monitoring - Testing performed continuously across the life of the installation. The intent is to monitor and confirm ongoing performance of the system:
  - Indicator organisms analyzed in the feed and filtrate.
  - Samples taken once per month for the 2nd year of operation.
  - Provided performance is stable, analysis can be reduced to quarterly for the 3rd and following years of operation.

A specific statistical analysis approach based on unpaired Monte Carlo analysis of feed and filtrate data was recommended for Tier 2 in (Salveson et al., 2021). Final LRV credits would then take the value of the 5th percentile demonstrated during pre-commissioning and commissioning. Reductions in performance during ongoing monitoring may require adjustment of ongoing LRV credits and/or trigger more frequent sampling of pathogens.

The intent of Tier 3 validation is to correlate LRV with a single or range of online monitoring techniques. As a result of the complex mechanisms of pathogen removal in MBR Tier 3 is hypothetical and has not yet been demonstrated. No published information that demonstrates Tier 3 is currently available, although several studies are ongoing or planned to develop this approach. Once a suitable Tier 3 monitoring solution could be demonstrated, future Tier 3 validation activities would be limited to confirming that initial correlations hold at new facilities and as a result may be broadly applicable to multiple MBR products or may remain product and application specific.

1.2 Regulatory Context

The California Division of Drinking Water (DDW) has finalized regulations for groundwater recharge and surface water augmentation (California Code of Regulations [CCR] Title 22 Division 4, updated October 1, 2018), two types of “indirect potable water reuse” (IPR) projects. DDW is developing regulations for “direct potable reuse” (DPR) projects, using terms such as “raw water augmentation” or “finished water augmentation”. For IPR projects in California, per Section 60320.108 and 60329.208 (SWRCB, 2018), a minimum pathogen log reduction target of

12-log enteric virus, 10-log *Giardia cyst*, and 10-log *Cryptosporidium oocyst*, from the point of raw wastewater to the point of water consumption is required; noting that requirements for surface water augmentation vary based on the percent by volume of recycled water delivered to the surface water (Section 664668.30) (SWRCB, 2018). For surface application and soil aquifer treatment, if recharge water already meets the definition of disinfected tertiary recycled water which is achieved by MBR + UV, it is likely that additional treatment would not be needed, but retention time underground would need to be demonstrated per §60320.108 (e) (SWRCB, 2018). The practice of setting overall LRV targets based upon end use is fundamental and applied in a number of jurisdictions within the US and internationally (NRMMC, 2006; WHO, 2017).

IPR treatment may include a range of technologies, depending upon how the reclaimed water is applied to the environment; with tertiary treatment (filtration and disinfection as defined in Title 22 sections 60301.320 and 60301.230) required for spreading and reverse osmosis (RO) and advanced oxidation process (AOP) required for direct groundwater injection and reservoir augmentation projects. For groundwater injection and reservoir augmentation, the treatment train must include at least three separate treatment processes. No single process can provide more than 6-log reduction, with at least three processes each being credited with no less than 1.0-log reduction (SWRCB, 2018). The compilation of technologies to meet the 12/10/10 rule is proven and replicable.

Table 1-1 presents the LRVs of an MBR-based reuse system according to the LRV credits proposed in (Salveson et al., 2021). Based upon Tier 1 LRV credits of 2.5 for protozoa, the listed treatment train marginally meets the regulated goals, whereas a more traditional microfiltration (MF) or ultrafiltration (UF) membrane would provide an additional 1.5 LRV credits of redundancy. Higher LRVs, via a Tier 2 system, would provide substantial value and operational flexibility to potable reuse systems, perhaps even allowing a reduction in subsurface travel time down to the 2-month minimum in California.

Table 1-1. LRV Across an MBR-Based Reuse Process Using the Tier 1 Default Credits.
(Data from Salveson et al., 2021)

Pathogens	Removal Goal (SWRCB 2018)	MBR	RO	UVAOP	Subsurface Travel Time	Total Credits
Viruses	12	1.0	1.5	6	6	14.5
Giardia	10	2.5	1.5	6	0	10
Cryptosporidium	10	2.5	1.5	6	0	10

1.3 Membrane Integrity

Historically, submerged membranes within MBRs have not been designed to perform and do not have the ability to withstand the daily pressure decay test (PDT) requirements specified in the USEPA Membrane Filtration Guidance Manual (MFGM) to correlate to a 3-micron size breach (USEPA, 2005). Most tertiary or drinking water MF and UF systems use this form of direct integrity testing (DIT) to demonstrate membrane integrity on a daily basis, thus allowing for 4-log removal credit (or greater) for protozoa. MF and UF systems used for potable reuse are typically not credited with virus removal as most states prefer to use the crediting

framework outlined in the MFGM which refers specifically to the use of PDT which does not have the resolution to verify virus removal. The industry has needed an alternative to the DIT framework, particularly to justify pathogen LRVs for MBRs, but there has been insufficient evidence available in the literature to support a specific correlated surrogate. However, evidence is now starting to accumulate, as documented herein.

By design, MBRs are subject to harsher conditions (higher total suspended solids [TSS] and other debris) than membrane filters placed after secondary treatment and clarification, resulting in increased damage and potentially greater difficulty in satisfying PDT requirements. Further, the caking of solids on the MBR, even after a backwash, are presumed to mask membrane breaches that may only show up after MBR chemical cleans, which are not performed on a daily basis. Overall, the industry concern about MBR reliability as a pathogen barrier is that they may have membrane or system integrity (i.e., connection hoses, module frames) failures that go unnoticed, which could result in partial loss of a pathogen barrier under some operational conditions. However, research has shown that MBRs, both flat sheet and hollow fiber, both new and old, and even when intentionally damaged, are able to maintain robust pathogen removal (Fontaine and Morris, 2020; Salveson et al., 2021; SCVWD, 2017) as long as regulated turbidity values are being met. This is the basis behind the potentially conservative Tier 1 and Tier 2 frameworks.

To increase the frequency that pathogen LRVs can be verified there is a need to identify surrogates that correlate with pathogen reduction, which is the focus of this Tier 3 project. Ideally, surrogates could be measured in real time, or at least a frequency sufficient to protect public health. Said another way, this ***Tier 3 aims to identify a metric(s) that is indicative of system integrity and changes with a resolution that can identify and accurately reflect any potential change in pathogen LRVs.***

1.4 Project Goals

The goals of this project were to:

- Evaluate the proposed WaterVal Tier 3 approach, considering how to correlate pathogen LRV and a monitoring system result.
- Survey utilities and suppliers to best understand the relative value of a Tier 3 system compared to Tier 1 and Tier 2 systems.
- Develop recommendations for how a Tier 3 protocol should be designed.
- Develop a U.S.-based MBR Validation Protocol Outline for Tier 3.

CHAPTER 2

Evaluation of Tier 3 WaterVal MBR Validation Protocol

The WaterVal Tier 3 validation concept was intended to align with the Hazard and Critical Control Point (HACCP) risk management strategy for controlling health risks due to pathogens. HACCP was initially developed for control of food safety for astronauts and is now an accepted international standard for maintaining food hygiene across the supply chain (ISO 22000, 2018). HACCP has since been applied to water reuse, with an early conceptual focus in the Australian Guidelines for Recycled Water (NRMMC, 2006), the WHO potable reuse and drinking water guidelines (WHO, 2017) and also in recent CA DDW considerations for DPR (SWRCB, 2019). HACCP requires identifying a significant barrier to a hazard-the CCP and then implementing a monitoring strategy to verify in real time that the CCP is reducing the hazard to target levels (Walker et al., 2016).

When pairing monitoring techniques with a CCP, it can be beneficial to understand the removal mechanisms responsible for hazard mitigation and how particular monitoring techniques can verify the efficacy of particular removal mechanisms. One of the best examples in water recycling is monitoring of UV dose to verify process integrity and estimate pathogen reduction. In this scenario:

- The CCP is the UV disinfection system.
- The pathogen inactivation (or reduction) mechanism is generally nucleic acid damage due to photolysis.
- Pathogen LRV is assumed to follow a linear relationship with UV dose, as determined from laboratory-scale collimated beam systems and demonstrated in large-scale treatment systems.

The UV dose delivered in the field can be determined using a number of equations (accounting for hydraulics) in conjunction with system-specific parameters which can be monitored in near-real-time. These include UV transmittance of the water matrix, flow rate, lamp power, and UV intensity (Wright et al., 2020). The reactor specific characteristics of UV dose can also be determined by challenge testing with spiked organism and modelling the organism reduction as a function of operational parameters and the water quality specific impacts can be assessed using standardized collimated beam testing. Challenge testing is logistically challenging for larger systems where spiking target organisms can be expensive. Determining the equivalent UV dose by monitoring system-specific parameters is analogous to the ideal intent of a Tier 3 protocol for MBRs.

In this section, the existing WaterVal Tier 3 protocol is reviewed, and a gap analysis is performed. Prior to the review and gap analysis, it was considered necessary to conduct a short review on: (1) pathogen removal mechanisms and (2) monitoring techniques that are currently available to MBR with an aim of identifying promising candidates and approaches for further Tier 3 validation work.

2.1 Pathogen Removal Mechanisms in MBRs

Notwithstanding discrepancies in terminology used in the literature, there are four primary mechanisms of removal within an MBR: (1) size exclusion by the membrane, (2) entrainment within and rejection by the dynamic membrane fouling layer, (3), adsorption to activated sludge flocs, and (4) biological degradation (Hai et al., 2014). Adsorption to the membrane may also play a role, but at scale it is difficult to systematically account for adsorption over size exclusion by a clean membrane. The mechanisms are illustrated in Figure 2-1 below and elaborated on in the following section.

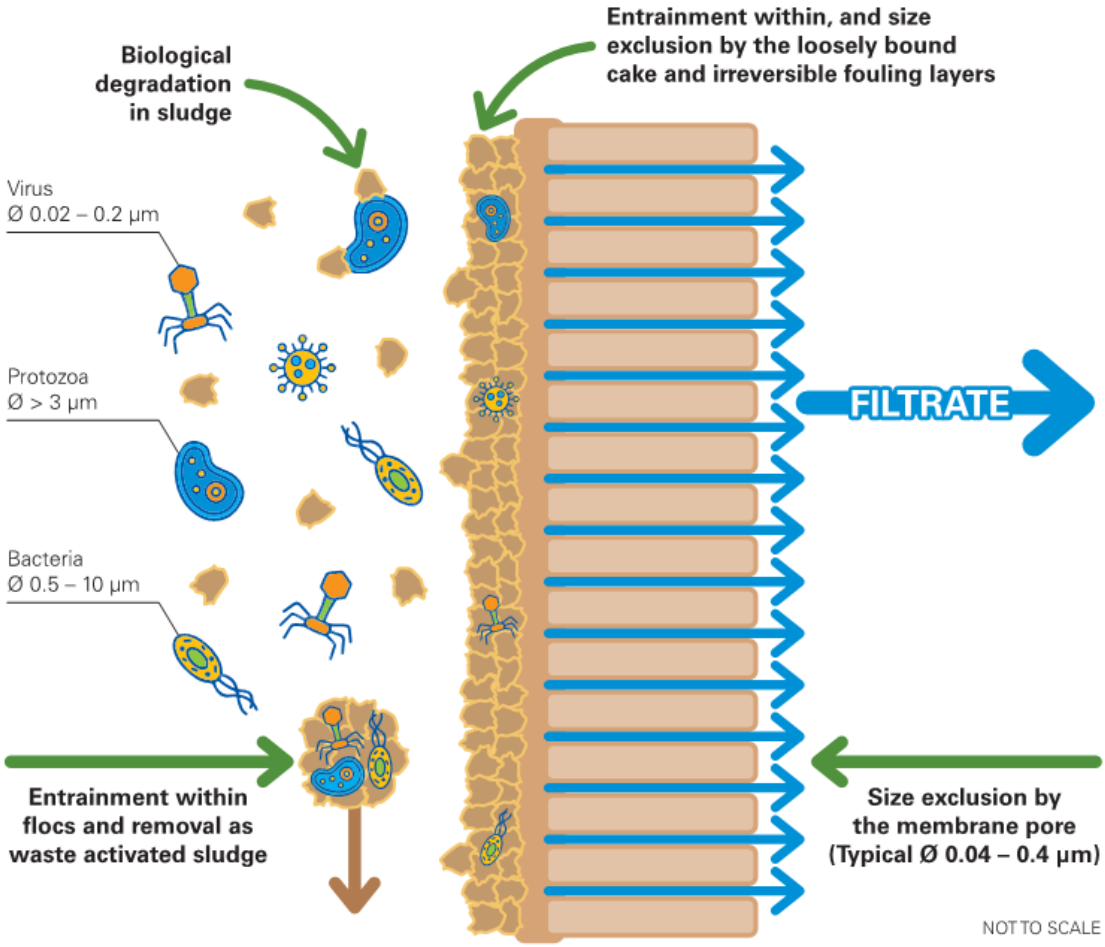


Figure 2-1. Illustration of Pathogen Removal Mechanisms in an MBR.

2.1.1 Size Exclusion by the Membrane

Assuming the membrane and module integrity is sound, size exclusion by the membrane itself is responsible for the rejection of any particle or pathogen larger than the pores of the membrane. UF and MF membranes exhibit respective pore sizes of 0.002 - 0.05 µm and >0.05µm (Judd, 2011). Typical commercial MF membranes do not have pore sizes exceeding 0.5 µm. Bacteria are typically sized between 0.5 - 10 µm; while viruses are typically 0.01 - 0.2 µm and protozoa are typically 3 - 14 µm (Antony et al., 2012). Salveson et al., 2021 recommended enteroviruses (0.025 - 0.03) as the target virus and *Cryptosporidium* (4 - 7 µm) as the target protozoa for MBR validation (Salveson et al., 2021).

In a full-scale study, the bacterial indicators enterococci, total coliform bacteria, and *E. coli* displayed LRVs > 5 log across the membrane. The site was equipped with KOCH MBR HF membranes with a reported nominal pore size of 0.1 µm, which would class them as MF. Removal from sewage to the mixed liquor was less than 0.5 log (Van den Akker et al., 2014). The results indicate that the contribution of size exclusion and enhanced rejection due to the fouling layer is expected to play a major role in removal efficiency, but that other mechanisms are also important. The importance of membrane integrity was acknowledged in Tier 2 validation protocols developed as part of Salveson et al., 2021, with the inclusion of a secondary technique to verify that the membrane was intact.

2.1.2 Rejection by the Fouling Layer

Adsorption of pathogens to the membrane, the cake layer (formed by the accumulated foulants on the membrane), or the biofilm (developing over filtration periods) has been cited as a contributing mechanism to explain appreciable rejection of viruses, which are typically smaller than the membrane pore size and are not expected to be removed by size exclusion alone (Ottoson et al., 2006; Shang et al., 2005; Sima et al., 2011; Ueda and Horan, 2000).

It is important to note that adsorption is not a permanent bond and desorption can occur. Additionally, once all potential adsorption sites are saturated, no further adsorption is expected to occur, so this can be a time-varying removal mechanism. A peak adsorption was observed for a fouled MF membrane operating at different fluxes (Farahbakhsh and Smith, 2004). As flux increased, coliphage retention increased to a maximum value and then started to decline. It was hypothesized that the corresponding increase in shear rates through the biofilm caused release of coliphage, captured by the biofilm, resulting in an overall decrease in rejection. In another experiment with 0.22 µm membranes filtering poliovirus (0.028 - 0.030 µm) in clean water, a peak virus rejection was observed on start-up, followed by decline to a minimum and then slow recovery. The rejection decline was attributed to saturation of sites capable of virus adsorption, while the recovery was attributed to build-up of deposits on the membrane (Madaeni et al., 1995).

2.1.3 Absorption by the Activated Sludge

The mixed liquor suspended solids (MLSS) component within the activated sludge presents another source of sites for adsorption of pathogens. Pathogens adsorbed to MLSS are anticipated to be removed from the MBR as the sludge is wasted to control solids retention time (SRT).

Adsorption of viruses to biomass and subsequent removal as waste activated sludge (WAS) was quantified through the use of molecular microbial analysis techniques (Sima et al., 2011). In a long-term study, increasing norovirus concentration in the WAS correlated with an increasing concentration of the wastewater and aeration basin concentration, but was delayed by the time equivalent to the applied SRT. From another full scale MBR, approximately 1000 times more norovirus GII was present in settled solids when compared with supernatant of the activated sludge (Simmons et al., 2011); Similar observations for norovirus, (i.e., 10 times higher detected associated with solids when compared to the supernatant) was observed within the

MLSS during another pilot scale study (Oota et al., 2005). Adenovirus and enterovirus concentrations were reported to be 100 - 10000 times higher for a settled portion of MBR activated sludge when compared with the supernatant. This indicated a similar affinity for adenovirus and enterovirus to adsorb to suspended solids and be rejected by the membrane (Kuo et al., 2010; Simmons et al., 2011).

2.1.4 Biological Degradation

In the activated sludge of a MBR, biological degradation is used to describe the inactivation of pathogens both by predation by larger protozoa and metazoans as well as decay. Biological degradation of bacteria was observed to be the more dominant removal mechanism, when compared to protozoa, in a study of conventional activated sludge (CAS). Protozoa in the CAS system were more dominantly removed via adsorption to biomass (Wen et al., 2009). Similar behavior of the activated sludge of an MBR system is likely to occur. CAS systems were attributed with an LRV for bacteriophages of 0.75 (Rose et al., 1996), which may have included adsorption and wasting. However, the biomass of an MBR was attributed with an LRV of 0.8 for MS2 phage, which corresponded to the LRVs reported for CAS systems (Shang et al., 2005). During a T4 phage spiking experiment, less phage was detected in the biomass of lab scale MBR with zero sludge wastage. It was suggested by the authors that a mixture of degradation and adsorption to the membrane gel layer was responsible (Lv et al., 2006); unfortunately, the relative effect of each, bio-degradation vs adsorption to biomass, was not quantifiable. Adenovirus and enterovirus concentrations in the activated sludge of an MBR were observed to be higher than influent wastewater by approximately 2 log, indicating a limited effect of biological degradation at reduction of these viruses (Kuo et al., 2010; Simmons et al., 2011). Unfortunately, insufficient data was presented to investigate how SRT, hydraulic retention time (HRT) and recycle ratios may have influenced sample results. Conversely, the norovirus GII concentrations of MBR influent and mixed liquor were equivalent to 1 log lower, indicating possible biological degradation (Simmons et al., 2011). With pathogenic viruses, a large number of investigations have used PCR based analyses. Limited studies have performed infectivity assays as well as PCR based quantification. To that end, there is uncertainty with regards to whether the apparent changes (increase or decrease) in the concentration of norovirus and other viruses are in-fact related to recovery issues or analytical limitations.

Cryptosporidium and *Giardia* have been observed to accumulate in the order of 1 log for a full scale MBR (Pettigrew et al., 2010), likely due to their high resistance to biological treatment as spore forming protozoa. Similar observations for accumulation of *Cryptosporidium* and *Giardia* were observed in the bioreactor at two different sites studied during the WaterVal project (Branch et al., 2021).

2.2 Sampling for LRV Calculation

The sample locations for a typical MBR configuration are shown in Figure 2-2 below.

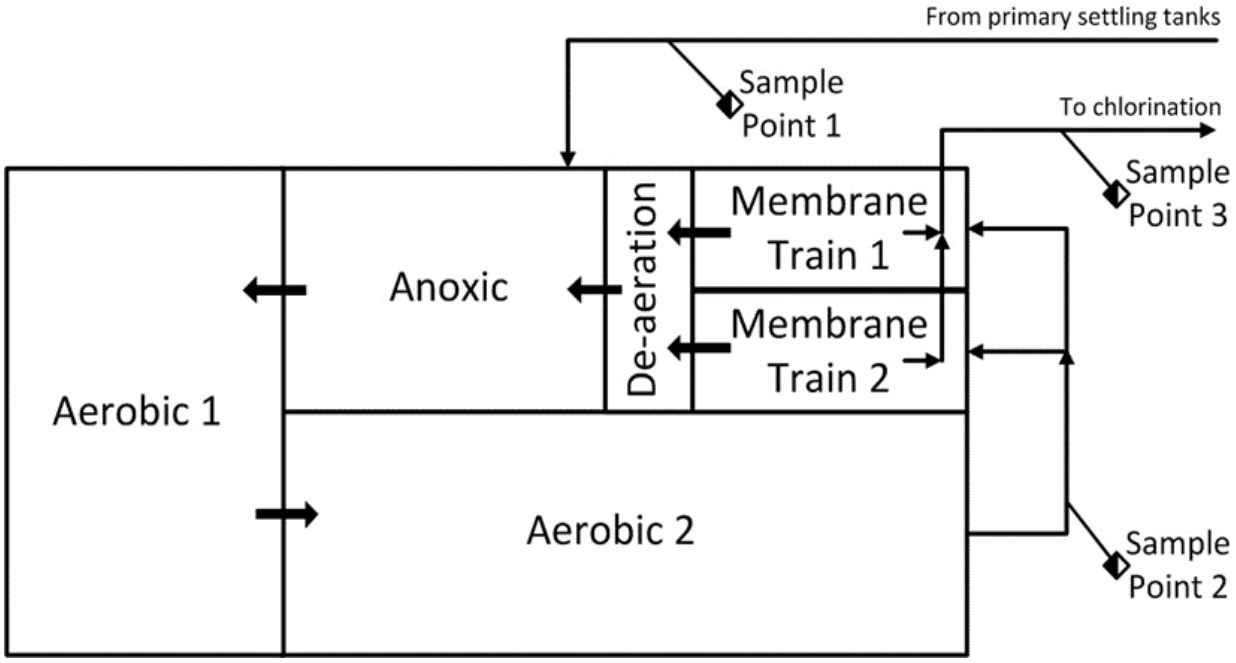


Figure 2-2. Sample Locations Around an MBR.
Sample Point 1 - MBR influent, Sample Point 2 - Activated sludge (MLSS) entering the membrane tank, and Sample Point 3 - MBR filtrate.

If samples of influent wastewater, activated sludge and filtrate are assayed, three different LRV can be calculated, the overall removal - LRV_{MBR} (Equation 2-1), the bioreactor removal - LRV_{Bio} (Equation 2-2) and the LRV across the membrane - LRV_{Mem} (Equation 2-3). LRV_{MBR} represents overall process removal by comparing influent and filtrate microorganism densities. LRV_{Bio} compares influent and mixed liquor densities and is representative of removal due to biological degradation only, provided a well-mixed sample of activated sludge is analyzed with a culture-based technique that can identify organism viability. In some past work, PCR based analyses have been performed which may underestimate degradation of organisms when calculating LRV_{Bio} (Simmons et al., 2011). Also, if only the supernatant of the activated sludge was analyzed, then the activated sludge density would also be lowered due to particle adsorption. LRV_{Mem} considers mixed liquor and filtrate densities and is representative of removal of microorganisms by the membrane and fouling layer.

$$LRV_{MBR} = \log_{10}\left|\frac{C_{In}}{C_{Filtrate}}\right| \quad \text{(Equation 2-1)}$$

$$LRV_{Bio} = \log_{10}\left|\frac{C_{In}}{C_{ML}}\right| \quad \text{(Equation 2-2)}$$

$$LRV_{Mem} = \log_{10}\left|\frac{C_{ML}}{C_{Filtrate}}\right| \quad \text{(Equation 2-3)}$$

Where C_{In}, C_{ML} and $C_{Filtrate}$ were the microorganism densities in the influent, mixed liquor (activated sludge), entering the membrane tanks and filtrate, respectively.

Depending on the nutrient removal goals of an MBR, there may be a number of recycle streams. Figure 2-3 below shows a possible MBR configuration for the purposes of nutrient reduction with example recycle streams.

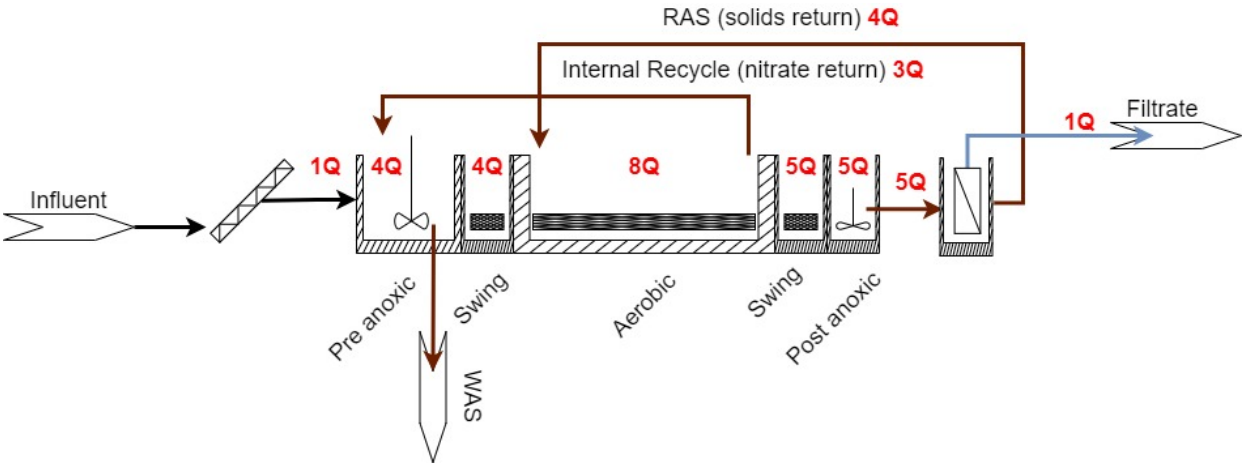


Figure 2-3. Recycle Flows in an Example MBR Configuration.
Single pass flows are shown as a multiplier of feed flow (Q).

Note that in the example shown, an internal recycle is used to return nitrate to the pre-anoxic zone to promote denitrification. A recycled activated sludge (RAS) line is used to return solids from the membrane compartment to the aerobic zone. There is limited information as to exactly how recycle rates and effective retention time in each zone may influence biological degradation. However, the higher flowrates through individual bioreactor zones is one factor that contributes to MBRs being modelled as complete mix systems. Under these conditions, an average concentration is anticipated to prevail.

Recycle flows through the membrane compartment are expected to influence the amount of pathogens accumulating in that reactor, i.e. a higher RAS will result in a lower volumetric concentration factor. The volumetric concentration factor for the bioreactor and membrane compartment was generalized in previous publications that investigated total suspended solids monitoring as a means to verify LRV (Katz et al., 2018). The resulting model is shown in Equation 2-4 below. The example in equation 2-4 conservatively assumes that there is no biological degradation and is essentially a particle material balance within the reactor.

$$LRV_{MBR} = LRV_{Mem} - \log_{10}\frac{SRT}{HRT} - \log_{10}\frac{1+RAS/Q}{RAS/Q} \qquad \text{(Equation 2-4)}$$

Where LRV_{MBR} is the overall LRV, LRV_{Mem} is the LRV across the membrane, RAS/Q is the ratio of the total filtrate flow to the recycle ratio from the bioreactor to the membrane tank, SRT = solids retention time in the bioreactor and HRT = hydraulic retention time.

If looking to sample to calculate LRV_{Bio}, the recommended location would be on the activated sludge line that feeds the membrane tank as this would provide the most well mixed sample

that has not been concentrated. Alternatively, the RAS leaving the membrane tank could be sampled but the volumetric concentration factor (VCF) of the membrane compartment would have to be accounted for.

2.3 Monitoring and Control Parameters for MBR

In this section, typical monitored and controlled parameters and their potential relationship with LRV is described. The aim of this section is to link parameters with removal mechanisms in an effort to clarify Tier 3 validation approaches.

2.3.1 Control Parameters for MBR

In general, each of the parameters described in this section can either be controlled during operation or design of an MBR facility.

2.3.1.1 Flux, Transmembrane Pressure, and Permeability

Flux is the flow per unit area of installed membrane. Most full scale MBRs are operated in constant flux mode, with a variable speed filtrate pump withdrawing a certain design flow rate. Although not typical, the exception are constant pressure MBRs, where the driving force for filtrate flow is provided usually by the hydrostatic head in the membrane tank and a control valve on the filtrate is used to regulate flow. Conflicting observations have been made regarding the influence of flux on LRV, with some sources reporting a decrease in rejection upon initial flux increase, followed by recovery (Farahbakhsh and Smith, 2004; Madaeni et al., 1995). The most common explanation is that following the increase in flux, the resultant shear disrupts the existing fouling layer and allows for greater pathogen passage across the membrane. Eventually, a new fouling layer forms, and the LRV returns to a nominal value.

When evaluating the removal of indicator organisms, a high flux has been shown to correspond with lower LRVs for *C. perfringens* and somatic coliphages but did not show a significant correlation for total coliforms or E. *coli*. Unexpectedly, transmembrane pressure (TMP) was negatively correlated and permeability was positively correlated with LRV - for total coliforms and *E. coli*, implying that a more fouled membrane exhibited a lower rejection (Branch et al., 2021). *C. perfringens* rejection across the membrane could not be significantly correlated with TMP or permeability. Rejection of somatic coliphages did show a significant negative correlation with permeability, but not high TMP. In a previous study, lower rejection of antibiotic resistant bacteria was observed at increased TMP and moderate fouling and then stabilized when membranes were critically fouled (Cheng and Hong, 2017). To better understand the impact of flux and permeability, a more significant data set at scale with a wide variation of the target operational parameters on a single system would be beneficial.

This complex behavior of indicator organism removal resulted in the WaterVal recommendation for challenge testing under various operational conditions. This is because removal does not always adhere to straightforward mechanistic principles, particularly across different types of organisms. However, removal by MBR can generally be described as follows:

- Clean membranes with high permeability and low TMP may achieve enhanced bacteria removal but lower virus removal - there was no significant correlation with *C. perfringens* as a surrogate for protozoa,
- MBR operation under high flux conditions may lead to lower virus and protozoa removal but may not have a significant impact on bacteria removal,
- Rapid increases in flux and disruption of the fouling layer may lead to decreased removal of indicator organisms until the fouling layer is reestablished.

2.3.1.2 Hydraulic Retention Time

Due to high aeration intensity and recirculation flow rates, the activated sludge compartment of an MBR approaches that of a 'completely mixed' system. Previously, researchers have applied pseudo first order kinetic modelling to the degradation of virus indicators in MBRs, in order to predict bio-predation (Chaudhry et al., 2015b, 2015a; Wu et al., 2010).

For a completely mixed system, the density of a microorganism degraded according to first order kinetics can be determined by Equation 2-5 (Wu et al., 2010).

$$C_{ML,t} = \frac{C_{ML,0}}{1+HRT.k_b} \qquad \text{(Equation 2-5)}$$

Where $C_{ML,t}$ and $C_{ML,0}$ are current and initial microorganism densities, respectively. k_b is the pseudo first order rate constant.

For a constant feed microorganism density (C_{In}), Equations 2-2 and 2-5 can be combined into Equation 2-6 to represent the LRV due to biological degradation (LRV_{Bio}) as a function of HRT.

$$LRV_{Bio} = \log_{10}\left|\frac{C_{In.}(1+HRT.k_b)}{C_{ML,0}}\right| \qquad \text{(Equation 2-6)}$$

Inspection of Equation 2-6 suggests that HRT is positively correlated with LRV (i.e., a longer HRT should result in a higher degree of biological degradation). Although a useful trend is presented, there will likely be difficulty in application of this model to full-scale data. Firstly, $C_{ML,0}$ would be difficult to determine, due to highly variable feed concentrations and the need to account for bioreactor recycle stream concentrations appropriately. In addition, diurnal temperature variations would likely affect the value of k_b throughout the day. As a guide, the k_b values may be useful in determining susceptibility to biological degradation (i.e., the higher the k_b value, the higher the LRV due to biological degradation for a particular organism). The k_b data from relevant references were combined in Table 2-1.

Table 2-2. Kinetic Data for Biological Degradation of Viruses in MBR.

Virus	k_b (h^{-1})	Reference
Wild Somatic coliphages	0.5	(Wu et al., 2010)[3]
Wild Adenovirus	0.4	(Chaudhry et al., 2015b)[2]
Wild Norovirus GII	1.3	
Wild male-specific coliphages[1]	2.0	
Lab grown MS2 bacteriophages[1]	0.22	(Chaudhry et al., 2015a)[3]
Lab grown phiX174 (a somatic coliphage)	0.13	
Lab grown male-specific coliphages[1]	0.07	

Notes:

1. Although MS2 is a male specific coliphage, results have been distinguished as there may be a diverse range of coliphage that are detected when analyzing environmental samples that include any coliphage present capable of replicating by infection of *E. coli* famp (ATCC: 700891).
2. The decay rate in this study may be an overestimate as a result of non-detect male-specific coliphages. Also, the pathogen decay constants may be an underestimate as they result from PCR based analyses. Decay results were modelled to account for recycle flows.
3. Studies were performed at bench scale with no recycle flows.

From the k_b values presented, it would appear that the lab grown bacteriophages exhibit a lower susceptibility to biological degradation, with lower rate constants than that of wild type viruses. The authors explained that the lab scale unit may have not operated as efficiently as a full scale MBR, and as a result, the rate constants presented may be conservative. Somatic coliphages appear to be more resistant to biological degradation, displaying lower rate constants when compared with wild male-specific coliphages (Chaudhry et al., 2015b; Wu et al., 2010), however, lab grown species appear to exhibit a similar rate of decay (Chaudhry et al., 2015a). In addition, the rate constants for somatic coliphages are lower than for norovirus GII and similar to adenovirus, which may indicate that they are more suitable than male-specific coliphages as a conservative virus removal indicator (Chaudhry et al., 2015a), with respect to degradation in the bioreactor. It is uncertain how effectively the PCR based analysis of adenovirus and norovirus accounted for biological degradation and the authors noted that the fitted decay rates for these species may be underestimates (Chaudhry et al., 2015b).

Fifth percentile LRV_{Bio} for wild somatic coliphages and wild male-specific coliphages assessed during the WaterVal project were -1.7 and -0.7, which is in agreement with the higher degradation rate of male-specific coliphages noted in Table 2-1 (Branch and Le-Clech, 2015).

The trend suggested by Equation 2-6 was also confirmed by correlation of a large set of indicator microorganism data collected during the NatVal study (Branch et al., 2021). As a result, it was suggested that operation at lower HRTs would likely lead to observation of more conservative removals.

For Tier 3 validation, systematic measurement of k_b values and modelling may be an appropriate means to consider HRT quantitatively as a bioreactor performance monitoring

parameters. To date, no k_b values have been reported for bacterial and protozoan pathogens or indicators in MBR.

2.3.1.3 Solids Retention Time

Effective size exclusion of larger pathogens and consequent retention in the bioreactor has the potential to result in accumulation in biology. This effect is magnified with longer SRTs due to greater flows of mixed liquor being recycled within the system rather than sent to waste. If system integrity is lost, there is potentially greater health risk due to passage of mixed liquor containing a higher concentration of pathogens. Utilizing a mass balance approach and assuming no biological degradation, the approximate concentration factor of these pathogens is described as the VCF, which is the ratio of the SRT to the HRT (Equation 2-7) (Katz, 2020).

$$VCF = \frac{SRT}{HRT} \qquad \text{(Equation 2-7)}$$

In independent studies (Marti et al., 2011; Van den Akker et al., 2014), similar findings regarding the densities of *E. coli* and *C. perfringens* in influent wastewater versus activated sludge of full scale MBRs have been reported. Specifically, a negligible difference in the concentration of *E. coli* in the influent wastewater relative to the activated sludge was observed. Whereas *C. perfringens* was detected in the bioreactor at concentrations approximately 1 log higher than the influent wastewater. The same observations were confirmed for *E. coli* and *C. perfringens* during the WaterVal project (Branch and Le-Clech, 2015). This can be partially explained by the propensity of *C. perfringens* to form spores (i.e., one of the reasons it is chosen as an indicator for *Cryptosporidium*). The spores display increased resistance to biological degradation (lower k_b). This example highlights that different mechanisms will be responsible for removal, depending on the nature of the pathogen or surrogate parameter.

Based on the results collected during WaterVal, SRT was positively correlated with LRV_{Bio} for total coliforms, *E. coli*, and somatic coliphages and not significantly correlated for male-specific coliphages and *C. perfringens* - suggesting that increased SRT favored removal which was not expected. It was previously thought that SRT should correlate negatively with LRV_{Bio}, as at longer SRTs greater accumulation of microorganisms should occur in the bioreactor, assuming no biological degradation (Van den Akker et al., 2014). This discrepancy may be due to the more dominant positive impact of HRT on biological degradation or potential for different activities of organisms at long vs. short SRT, than the possible negative impact from accumulation (Branch et al., 2021).

2.3.1.4 Mixed Liquor Suspended Solids

MLSS concentration will change as a result of organic, nutrient (biochemical oxygen demand, ammonia and phosphorous) and TSS loading levels in the feedwater, temperature, and VCF of the reactor. After some period of acclimation, MLSS should approach steady state values, which can be controlled by SRT adjustments.

MLSS is anticipated to hold a complex relationship with pathogen removal. Firstly, a higher MLSS would be anticipated to benefit pathogen removal as there is more chance for adsorption and shielding of membrane defects. When operating two submerged pilot reactors in parallel,

one with activated sludge and the other with raw wastewater only, a lower LRV was observed for the reactor without MLSS for a range of indicator microorganisms, until the membrane fouled significantly, which improved rejection (Branch et al., 2016). Secondly, for a higher MLSS it could be reasonably assumed that a membrane with integrity defects would allow a greater amount of TSS to pass the membrane and be more easily detected by a turbidity meter. Finally, a higher MLSS concentration is anticipated to correlate with a higher concentration of predators, thus a higher rate of biological degradation.

However, MLSS was significantly negatively correlated with LRV_{Bio} for *C. perfringens*, *E. coli* and somatic coliphages (i.e., a high MLSS was observed with lower LRV_{Bio}). In addition, *C. perfringens* and somatic coliphage activated sludge densities were positively correlated with MLSS, suggesting that these organisms accumulated via similar mechanisms to the MLSS. The correlation of a higher MLSS corresponding to lower observed overall LRVs was also significant for overall removal by the MBR for *C. perfringens* and somatic coliphages (Branch et al., 2021). Accordingly, the MLSS may be a more useful surrogate parameter, when compared to SRT, for monitoring the potential for accumulation of organisms within the MBR.

The MLSS was expected to follow the same trend as SRT as these factors should be proportional. However, not all sites operated with the same nutrient loading and as a result sludge yield at the same SRT would not necessarily have been equivalent. In addition, the ratio of HRT to SRT was not always equivalent, depending on system sizing. The MBRs evaluated during the NatVal study appeared to have HRT and SRT that were longer and MLSS and Flux on the lower range when compared to typical design parameters. Further pathogen removal studies at full scale installations would be beneficial to better understand the impact of HRT, SRT, Flux, MLSS, membrane configuration and material on LRV. In addition, use of combined variables or multivariate analysis may improve understanding of how operational parameters influent LRV.

2.3.2 Monitoring Parameters for MBR

Each of the parameters described in this section is a technique that could be applied for monitoring MBRs. Note that not all parameters specified are real time, but, could realistically be performed daily - meeting the minimum frequency criteria established in the MFGM for DITs and also corresponding to the daily health risk target recently adopted for DPR in California.

2.3.2.1 Turbidity

Turbidity is a measure of 'cloudiness' of water. Turbidity via measurement of light scatter at 90, known as nephelometry, is common in the water industry. Due to its convenience, filtrate turbidity measurements are one of the most commonly employed integrity monitoring strategies for MBRs (Judd, 2011). The resolution of permeate turbidity monitoring in membrane systems can be increased by moving from conventional to laser scattering systems, capable of reading to 1 NTU. It is noted that turbidity measurement features lower cost, but also lower sensitivity when compared to particle counting (Guo et al., 2010). In a pilot scale experiment, a laser turbidity unit was capable of detecting 1 cut fiber out of 300, when challenged with latex microspheres and MS2 bacteriophage. The loss in integrity resulted in a drop in MS2 bacteriophage LRV from 3.2 to 1 and a rise in permeate turbidity above the maximum range of

the turbidity meter (Mosqueda-Jimenez et al,. 2011). Log removal distributions for virus and bacterial indicators were compared at pilot scale, for a turbidity lower than 0.2 NTU, 95% of LRVs measured for somatic coliphages and bacterial indicators (*E. coli*, total coliforms and fecal coliforms) were above 3.1 and 4.8, respectively (Mosqueda-Jimenez et al., 2011).

In order to address the sensitivity limitation posed by dilution of contaminated flow through a defect, a multiplexed turbidity system with one detector connected to multiple monitoring locations via fiber optic cable was employed (Naismith, 2005). Laser turbidity was reported capable of detecting 1 broken fiber out of 5000 in a pilot membrane UF module, when fed with water of 12 NTU (Banerjee et al., 2001); the turbidity of activated sludge generally exceeds 12 NTU, therefore challenging the system and potentially increasing sensitivity. Frequent calibration and maintenance checks are required to ensure accurate turbidity readings (Farahbakhsh et al., 2003).

Turbidity can be used as an online surrogate for TSS, and its function in monitoring MBRs would be to confirm that the combined rejection mechanisms of membrane barrier and fouling layer are functioning. It has been unclear as to how the breakthrough of TSS quantitatively correlates with the breakthrough of pathogens and whether there are circumstances where pathogen breakthrough occurs but TSS breakthrough does not. Unsurprisingly, a higher filtrate turbidity has been shown to correlate with lower LRVs when MBR data sets have been analyzed (Branch et al., 2021; Salveson et al., 2021), but there are insufficient pathogen data to develop quantitative relationships and usable models.

An approach to correlate turbidity, TSS, and pathogen breakthrough in MBR was proposed and evaluated for the WaterVal project and is described in Section 2.5.2.

2.3.2.2 Filtrate TSS

As an extension to turbidity, TSS has been monitored in the filtrate of MBRs and an equivalent LRV tracked across the process. Typically, TSS is below 5 milligrams per liter (mg/L) in MBR filtrate, which is the typical detection limit with standard filtration volumes. In order to obtain measurable quantities of TSS, larger filtrate volumes have been analyzed by using filters connected directly to the MBR filtrate.

An approach for overall calculation of an LRV based on filtrate TSS monitoring was described in previous literature (Katz et al., 2018). It has been proposed that an overall LRV could be calculated by subtracting the VCFs due to the bioreactor (Equation 2-7) and membrane tank and was determined using the relationship in Equation 2-8.

$$LRV_{TSS} = LRV_{Mem,TSS} - \log_{10}\frac{SRT}{HRT} - \log_{10}\frac{1+RAS/Q}{RAS/Q} \qquad \text{(Equation 2-8)}$$

Where LRV_{TSS} is the overall TSS LRV, $LRV_{Mem,TSS}$ is the LRV of TSS across the membrane, RAS/Q is the ratio of the total permeate flow to the recycle ratio from the bioreactor to the membrane tank.

Used in isolation, high volume TSS monitoring is not able to provide a measure of instantaneous membrane integrity due to the need to accumulate a detectable quantity of TSS on the filter.

However, high volume filtrate TSS measurements could realistically be performed daily, as they are simple and with the use of Nylon membranes the method appears to be robust. If operated at constant flow using a pump, the high volume TSS provides a time proportional composite indicator of the MBR filtrate quality over the sample collection period. In addition, if a feed side pressure transducer was incorporated into the TSS apparatus, then spikes in pressure across the filter could be used to infer an increase in solids loading - which may indicate integrity failure. At present there are no commercially available systems for high volume TSS analysis. But a high volume TSS monitoring system could be custom built from simple components and automated for sample collection. Filtrate TSS monitoring is conservative with respect to potential accumulation of pathogens in the bioreactor and does not account for biological degradation. Also, to date, there are no side-by-side results that have quantified pathogens or indicator organisms and attempted correlation with the TSS approach. Although conservative with respect to biological removal, the method was applied to collect a filtrate composite over 24 hours, over which time approximately 125 gallons (470 L) was filtered on average, and an LRV_{TSS} of 4.7 was demonstrated (Katz, 2020).

2.3.2.3 Pressure Decay Testing

PDT is well established in the USEPA MFGM for use in verification of protozoa removal by MF and UF membranes (USEPA, 2005). Due to the fact that a majority of MBR membrane systems are not equipped or are unable to perform PDTs without compromising membrane integrity and concerns that PDTs may be too conservative or lack correlation for verification of LRV in MBR, they were not included in the WaterVal protocol. PDTs are suspected to be conservative for MBRs as they can only account for size exclusion of the membrane and not the potentially significant contribution of the fouling layer, adsorption onto the mixed liquor solids and also biological degradation. In fact the WaterVal protocol said that should a utility wish to validate an MBR using PDT, it was simply recommended to follow the existing guidance in the MFGM (USEPA, 2005) as if the MBR was a MF/UF system (WaterSecure, 2017).

There are few examples of the long-term impacts of regular PDT on the integrity of MBR systems. In addition, there are now publicly available studies where either the pressure decay rate (PDR) or a calculated LRV has been correlated with LRV. In addition, there is uncertainty as to whether performing a PDT could disrupt healing mechanisms and the cake layer that provides additional removal at the membrane surface. That is, if PDTs were used and LRV was determined by sampling after PDT it is anticipated that the observed LRVs may be lower than if samples were taken from an identical system that was not performing PDTs (i.e., a system without disturbances to hollow fiber plugging and the cake layer). No study exists which has collected pathogen data of two side by side identical systems where one system was undergoing PDTs and the other was not. Notwithstanding the uncertainty above, it is anticipated that sample collection immediately following a PDT would represent a conservative condition for determination of MBR LRV and that for the period between PDTs, LRV may improve.

Nevertheless, PDTs are simple, requiring only a pressure transducer and compressed air source, which are typically available for certain configurations of MBRs. In addition, some form of PDT,

not necessarily in line with the USEPA MFGM from a type or pressure perspective, but sufficient to confirm overall membrane integrity, has been recommended as an option for additional monitoring as part of Tier 2 validation protocol and as a voluntary monitoring parameter as part of the Tier 1 validation protocol developed for Salveson et al., 2021. As a result, it is relevant to consider PDTs as a monitoring technique for Tier 3 validation.

When following the USEPA approach for calculating an LRV from PDT results, there are multiple operational parameters that need to be accounted for. The calculation of an LRV based on PDT is intended to document the minimum removal of protozoa by membrane separation only, not to account for other methods of pathogen reduction within the MBR. LRV calculated based on PDT is dependent on several membrane-specific and operational variables, thus this method is ideally more representative of membrane removal of pathogens compared to a PDR value in isolation. The variables involved in calculation of a PDT LRV, as well as their impact on LRV, are summarized in Figure 2-4.

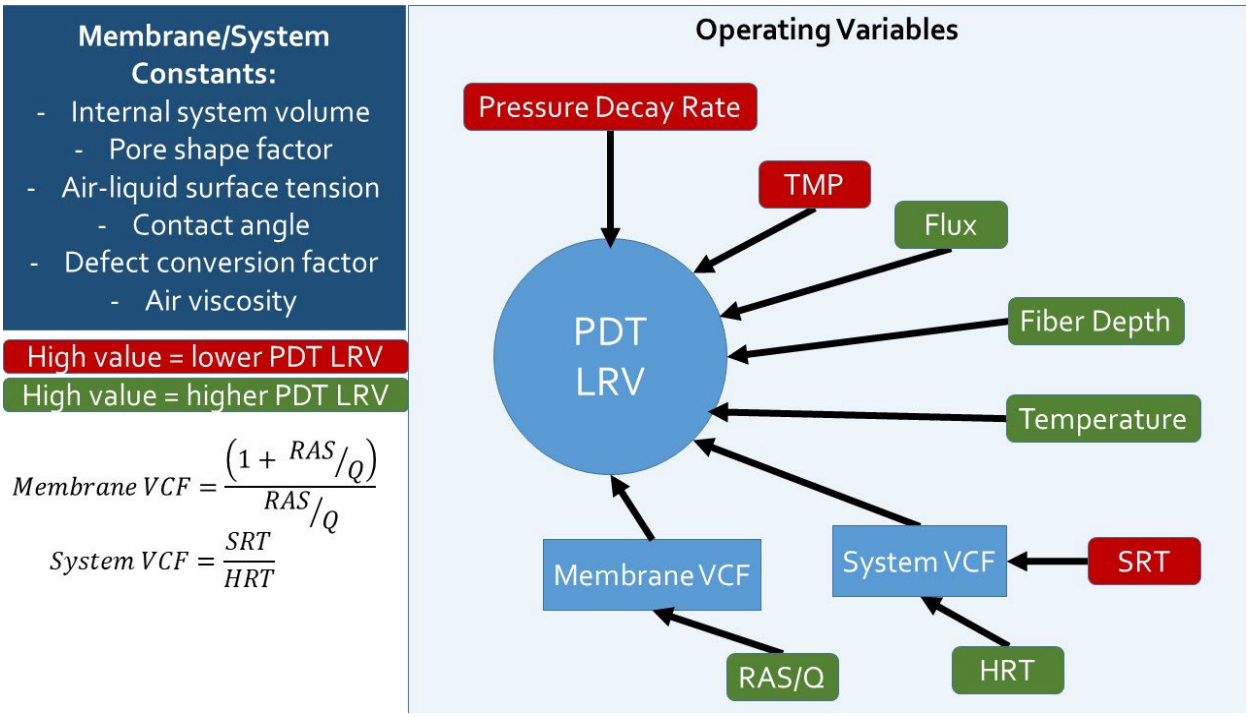


Figure 2-4. Relationship Between Operating Variables and Final Calculated PDT LRV.
Membrane and System volumetric concentration factors (VCF) result from a material balance assuming no biological predation. Note: Q - permeate flow, RAS - recycled activated sludge, SRT - solids retention time, HRT - hydraulic retention time and TMP - transmembrane pressure (see Equation 2-4).

When applying PDTs for LRV calculation in MBR, there are uncertainties as to where to set the boundaries for calculation (i.e., membrane tank only or whole bioreactor). These boundaries determine what value the VCF takes in the calculation of an equivalent LRV from PDT results. On top of these uncertainties, there are the same conservative elements that apply to the filtrate TSS monitoring, as the impact of biological degradation is not accounted for. In addition, a PDT will confirm the integrity of the membrane barrier and will likely underestimate the potential contribution of the dynamic fouling layer.

PDTs are not suitable for all types of MBR membranes. For example, the force distribution from pressurization of the filtrate side of a flat sheet membrane may concentrate and damage the glue lines that join the membrane to the module housing. Also, there is concern that when membrane damage does occur, repeated use of a PDT may push out any clogging or fouling that has sealed defects, effectively reducing the ability of the membrane to heal due to the fouling layer.

Although there are numerous data sets comparing PDT LRV and indicator microorganism challenge tests for MF/UF systems, there are minimal data sets comparing PDT and pathogen reductions in MBR. One data set showed that when PDT LRV dropped significantly after membranes were damaged by fiber cutting, the LRV of *Bacillus subtilus* remained high (Mosqueda-Jimenez et al., 2011). A similar observation was made during testing as part of the SCVWD, 2017 study, where PDT suggested LRV would be poor, but LRV was significant (SCVWD, 2017). For consideration of PDTs for Tier 3 validation, steps will need to be taken to address the potential level of conservatism of PDT LRVs when compared to LRVs derived via challenge testing. That is, it may not be appropriate to assume that the LRV calculated from PDT results aligned with the MFGM is the true, or even a mildly conservative indicator of removal. Instead, it is suggested that either the PDR or a calculated LRV should be correlated with the LRV of target pathogens and that this correlation would guide quantification of critical limits and the operational LRV (of pathogens) at those limits – which would serve as the log reduction credit.

2.3.2.4 Total Organic Carbon

Total organic carbon (TOC) removal across the system, influent to filtrate, was shown to hold a significant, but weak, correlation with biological degradation of organisms from the WaterVal data set. It may be possible to demonstrate a better site-specific correlation as a means to monitor bioreactor performance (Branch et al., 2021). It is possible that the TOC removal correlation with LRV_{Bio} was coincidental as TOC removal was indicating good activated sludge performance, which in turn may have resulted in higher degradation rates for indictor microorganisms.

TOC monitoring equipment is typically expensive ($20 - 40k), requires comparatively high maintenance, and is generally reserved for clean filtered water sources, such as monitoring removal by RO. While monitoring the MBR filtrate is achievable, monitoring influent wastewater online may result in challenges due to instrument fouling. There is potential to use other surrogates for TOC, such as UV transmittance (UVT), which for a relatively stable site-specific wastewater matrix, should yield a reasonable correlation with TOC. UVT meters are available in online configurations and are also cheaper than typical TOC units. Chemical oxygen demand (COD) may also correlate with TOC and could be used as a readily available offline test (taking approximately 3 hours), in lieu of online UVT or TOC.

It may be interesting to pursue analysis of TOC (or UVT, COD) removal as a surrogate for bioreactor LRV. Unfortunately, the data to date is limited, is only available for indicator microorganism and not pathogens.

2.3.2.5 pH and Conductivity

There have been limited studies looking at the impact of pH and conductivity on removal in MBR. However, both of these parameters would be expected to impact adsorption to both the membrane and activated sludge.

pH has been shown to affect both the zeta potential and hydrodynamic radii of viral indicators T4 coliphage and MS2 phage. The hydrodynamic radii of MS2 decreased from 170 to 15 nanometers (nm) over a pH range from 3 to 10, remaining below 20 nm from pH 5 to 10. Zeta potential measurements for MS2 reached 0 at a pH of 3; accordingly, aggregation of MS2 was attributed for the increase in size. T4 phage did not display the aggregation similar to MS2; however, the size of T4 did reduce from 140 to 60 nm for an increase in pH from 3 to 10 (Arkhangelsky and Gitis, 2008). Early work on MF membranes in clean water indicated higher rejection of virus indicators MS2 and T4 coliphage, exceeding 80% at pH 4. This was attributed to virus aggregation at low pH due to the change of surface charge, from negative to positive, when the pH decreased below the isoelectric point (IEP) of the viruses (Herath et al., 1999). There was a significant correlation between MS2 rejection (Herath et al., 1999) and hydrodynamic radii (Arkhangelsky and Gitis, 2008) reported in separate investigations. Below pH 5, MS2 radii and rejection increased very rapidly with decreasing pH values; while above pH 5, rejection and radii were stable at 20% and 20 nm, respectively. Normal pH of MBR was reported in case studies as 7 - 8 (Judd, 2011). Hence, decreases in pH to below the virus IEP (pH 3 - 5, (Michen and Graule, 2010)), promoting virus-to-virus interaction, could only be considered originating from extreme events. pH was positively correlated with LRV_{Bio} for *C. perfringens*, total coliforms, *E. coli*, male-specific and somatic coliphages, and the correlation with *C. perfringens*, total coliforms and *E. coli* was also significant for overall LRV based on the NatVal project data (Branch et al., 2021). This suggested that higher LRVs were more often observed at higher pH. However, the overall pH range of the sites studied was small, ranging from 5.5 to 7.5, and it is unknown whether pH was a contributing factor, or if it was just that sites that were adding alkalinity to promote nutrient removal were in general more stably operated.

The addition of 20 grams per liter (g/L) sodium chloride (NaCl) to a pilot MBR lowered the removal efficiency of male-specific coliphages from 5.4 logs before NaCl addition to 3.9 logs 1 day following NaCl addition (Branch et al., 2016). In previous research, increasing the ionic strength, from 0 to 0.02 M as NaCl, was shown to enhance membrane rejection (Van Voorthuizen et al.,2001). Other studies have reported that the adsorption affinity of bacteria increased up to ionic strengths of 0.1M. At ionic strengths greater than 0.1M, a reduction of adsorption affinity was noted relating to the molar concentration of cations (Stevik et al., 2004). On the addition of 20 g/L of NaCl, ionic strengths in the hazardous event experiments conducted in Branch et al. (2016) would have increased equivalent to 0.3M. The increase of the ionic strength to such high levels may have reduced the degree of virus adsorption to activated sludge flocs, and subsequent transfer across the membrane at a higher rate than adsorbed particles. The experiments in Branch et al. (2016) were conducted on pilot units to simulate potential hazardous events. There is only one reported instance of an MBR receiving a salinity shock of this magnitude, which occurred when Porlock MBR in the U.K. suffered from severe seawater ingress into raw wastewater pipes (Severn, 2003). Although

resulting maintenance issues were reported, there was not information available as to changes in virus rejection.

Monitoring of pH is typically practiced in MBRs for the purposes of optimizing nutrient removal. In water recycling schemes, filtrate conductivity of the MBR is likely monitored as feed to the RO. Significant changes of either of these meters may indicate an issue with MBR operations, are suspected to influence adsorption, but a significant impact to LRV is still yet to be demonstrated with monitoring of pathogen reduction data. In addition, it is unlikely that these parameters would vary to a significant enough extent at a single site to influent pathogen removal.

2.3.2.6 Indicator Microorganisms

Indicator microorganisms are not an ideal candidate for Tier 3 validation as there are presently no devices that can measure automatically in real time. However, as technology develops it may be possible to overcome this hurdle.

In Salveson et al., 2021, total coliforms and the trend in their filtrate concentrations are proposed as a secondary membrane integrity surrogate (Salveson et al., 2021). There are devices that can automatically measure total coliforms, *E.coli* or fecal coliforms in time periods less than 24 hours if the concentration is sufficiently high. In addition to strict culture-based/enzymatic activity systems, there are other variations of these systems available, and they have been reviewed (Demeter et al., 2020). There are also some possibilities to adapt similar systems for coliphage analysis as a means to verify virus removal. If a correlation with LRV could be developed using these devices, then automated monitoring of total coliforms or other microbial surrogates may be satisfactory for Tier 3 validation.

To date, there is limited information on how total coliform filtrate trends may correlate with pathogen removal, but this may change as more MBR systems follow the Salveson et al., 2021 Tier 2 validation protocol.

Other indicator microorganisms that may be surrogates could include those listed in Salveson et al., 2021, somatic or male-specific coliphages and *C. perfringens*. However, there are no existing automated devices to permit rapid and automated measurement of these organisms.

2.3.3 Monitoring Summary

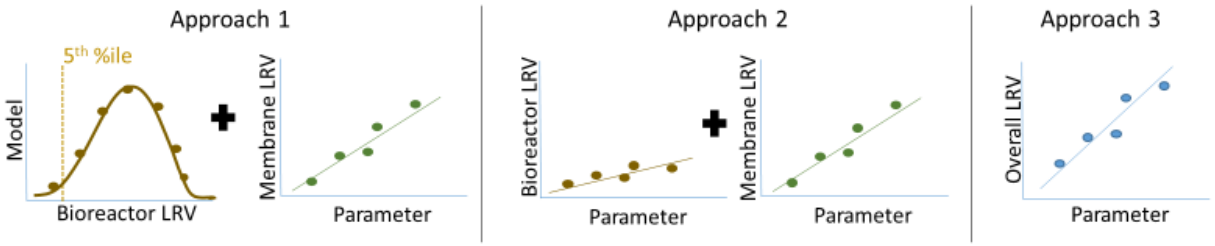
The range of techniques above show varying levels of readiness and promise for use in Tier 3 validation. For each technique, the capacity to monitor each removal mechanism is summarized in Table 2-2 below.

Table 2-3. Monitoring and Control Parameters and Removal Mechanisms in MBR.

Monitoring or Control Parameter	Size Exclusion by Membrane	Rejection by Fouling Layer	Biological Degradation	Adsorption to Activated Sludge
Flux, TMP, Permeability	Related	Indicated by permeability.	N/A	N/A
HRT	N/A	Possible secondary impact to fouling behavior.	Related by kinetic degradation. Impacts accumulation through VCF.	N/A
SRT	N/A	Possible secondary impact to fouling behavior.	Impacts accumulation through VCF. Secondary impacts through MLSS.	N/A
MLSS	N/A	Potential higher likelihood of shielding.	Potential higher organism population for biological degradation.	More sites for adsorption.
Turbidity	Indicates removal mechanism if TSS removal is equivalent to pathogen removal.	Indicates removal mechanism if TSS removal is equivalent to pathogen removal.	Not related	Adsorbed pathogens that transfer to the filtrate are likely detected by turbidity, but not related to mechanism.
Filtrate TSS	Indicates removal mechanism if TSS removal is equivalent to pathogen removal.	Indicates removal mechanism if TSS removal is equivalent to pathogen removal.	Conservatively accounted for by use of VCF.	Adsorbed pathogens that breakthrough to the filtrate likely detected by TSS, but not related to mechanism.
Pressure Decay Testing	Indicates removal based on particle size related to test pressure.	Does not account for fouling layer impacts.	Conservatively accounted for by use of VCF.	Does not account for adsorption.
Total Organic Carbon	Does not indicate.	May be indicative of enhanced rejection of larger dissolved organic compounds by fouling layer.	Shown to correlate with LRV_{Bio}. Uncertainty surrounding pathogen removal.	Unclear, may be indicative of enhanced rejection of DOC adsorbed to sludge.
pH and Conductivity	Possible secondary impacts based on hydrodynamic radii.	Possible secondary impacts to fouling development and hydrodynamic radii.	Possible secondary indicators of stable bioreactor performance.	Significant changes may indicate adsorption differences but not confirmed with pathogen data.

2.4 Approaches for Tier 3 Validation

Given the range of mechanisms of pathogen removal occurring in an MBR, it may be difficult to find one particular monitoring technique that can satisfactorily correlate with LRV. In this section, Tier 3 validation is separated into 3 different approaches that could be followed to achieve Tier 3 validation. The concept of each approach is illustrated in Figure 2-5 and defined below.



- Demonstrate Membrane LRV correlation
  - Proposed surrogates, turbidity, turbidity/TSS, PDT
- Validate bioreactor removal (similar to Tier 2) or
- Model bioreactor removal
- Add LRVs from bioreactor and online membrane correlation.

- Demonstrate Membrane LRV correlation
  - Proposed surrogates, turbidity, turbidity/TSS, PDT
- Demonstrate Bioreactor LRV correlation
  - Proposed surrogates TOC , UVT, COD removal
- Add LRVs from bioreactor and online membrane correlation.
- Uncertainty as to bioreactor monitoring parameters, limited TOC data and only correlated with indicators

- Demonstrate Overall LRV correlation.
- Proposed surrogate: indicator microorganisms or others shown to correlate with LRV
- No available data

Figure 2-5. Approaches for Tier 3 Validation of MBRs.

2.4.1 Approach 1 - Validate a Technique for Membrane LRV and Use a Tier 2 Style Empirical Approach to Define and/or Model A Bioreactor LRV

- There are a variety of proven membrane integrity monitoring techniques (e.g., PDT, turbidity, TSS monitoring).
- Approach 1 was suggested for use with turbidity in the WaterVal project and is discussed further in Section 2.5.2.
- Approach 1 has also been demonstrated with total suspended solids as defined in (Katz, 2020) and described in Section 2.3.2.2. However, pathogen or indicator data has not been collected alongside TSS rejection as a means to verify this approach yet.
- Approach 1 may be suitable for use with PDT as described in Section 2.3.2.3. However, as with TSS monitoring, a corresponding set of pathogen and indicator microorganism data has not yet been collected.
- This approach is somewhat justified as a majority of the LRV contribution, (i.e., hazard control) appears to occur across the membrane barrier for most organisms (Branch et al., 2020).

- If an extensive data set for pathogen removal by the bioreactor could be gathered, then it may be possible to propose default bioreactor LRV credits that would be used in lieu of monitoring or site-specific validation.
- If further bioreactor degradation studies could be conducted to ascertain K_b values for a wider array of pathogens then it may be possible to model bioreactor LRV based on HRT and recycle flows (see Table 2-1).

2.4.2 Approach 2 - Validate Separate Techniques for Verification of the Bioreactor LRV and Membrane LRV, the Sum of These LRVs Can Then Be Used to Verify Overall Removal

- This approach is more comprehensive as if successful it would account for all possible removals across the system.
- The NatVal data set which considered indicator microorganisms suggested that there was a significant relationship between TOC removal across the MBR process and bioreactor LRV (Branch et al., 2021). Given the success of TOC monitoring, it may be possible to expand this approach to include other surrogates known to correlate with TOC including UV transmittance or COD.
- With systems under stable operation, it may be possible to define kinetic parameters for biological degradation of pathogens as was performed in literature previously (Chaudhry et al., 2015b, 2015a). These models if successful and verified with monitoring data, may allow bioreactor removal calculated as a function of HRT, SRT and temperature.
- To demonstrate Approach 2, more systematic experiments covering a range of equilibrated HRT, SRT and temperature with real pathogens as well as indicator microorganisms would likely be required.

2.4.3 Approach 3 - Identify a Surrogate That Can Be Measured Online and Correlates with Whole System Removal

- There are limited available options at present that could realistically support Approach 3. A suitable microbial indicator organism that could be measured automatically or at a higher frequency might.
- Pepper Mild Mottle Virus (PMMoV) may be a promising indicator as it (Papp et al., 2020):
  - Is a plant virus and is not toxic to humans.
  - Is rod-shaped (18 × 312 nm) RNA virus with a low IEP (3.2 - 4.9).
  - Is abundant in wastewater.
  - LRVs through an MBR of >1.0 - 1.9 have been demonstrated when 10 L of filtrate were collected.
- Also, there are techniques under development that could allow for automated and high frequency analysis of PMMoV. The small diameter of PMMoV and low IEP suggest that it may be poorly removed by membrane filtration. However, the large rod length does create uncertainty. Nevertheless, even the length of the rod-shaped PMMoV is much smaller than most bacteria and protozoa and therefore PMMoV should still be a conservative surrogate for protozoa removal.

- If a correlation between PMMoV removal and LRV of target organisms could be demonstrated, and if automated instrumentation to provide high frequency data was sufficiently robust and reliable then it could be used as a surrogate for Tier 3 MBR validation.
- As noted previously, automated analysis of total coliforms and *E. coli* also exist and might provide a viable means of inferring LRV provided that removal of these indicators appropriately correlated with LRV. Other microbial surrogates may also be of interest, provided that technology is available to allow frequent and automated measurement and that the surrogates can be shown to correlate with pathogen removal.
- Alternatively, if sufficient data for other non-microbial techniques, such as turbidity or PDT, could be shown to effectively indicate LRV then they may be able to be used to set reliable control limits within which a conservative LRV can be granted.
- With further pathogen removal data, it may be possible to develop a combined operating variable (from multiple monitored parameters) that adequately describes LRV. However, to date this data set is not available.

2.5 WaterVal Review

WaterVal (WaterSecure, 2017) proposed the Tier 1, 2, and 3 concepts for MBR pathogen rejection and performance monitoring. The efforts related to Tier 1 and Tier 2 have been evaluated and summarized in Salveson et al. (2021).

Tier 3 validation for WaterVal was always intended to meet the requirements of the *Australian guidelines for water recycling* (NRMMC, 2006), as opposed to the Tier 2 method, which only demonstrates performance at discrete points in time and does not provide continuous performance monitoring.

There are distinct advantages of Tier 3 validation, when compared to lower Tiers including:

- Operational Flexibility - LRVs may be demonstrated within an extended operating range with improved critical control limits. This may result in higher system productivity overall, with less stoppages due to unnecessarily conservative alarms.
- Response Time - LRV will be verified frequently allowing more rapid corrective actions and better protection of public health. While this may not be necessary for IPR, it is of great importance for DPR where allowable failure response time is shorter.

2.5.1 WaterVal Tier 3 Intent

The intention of a Tier 3 monitoring protocol was to be able to continuously monitor a performance surrogate and to correlate this surrogate to the pathogen removal performance of the MBR. Based on the existing scientific literature available at the time of WaterVal, no generic surrogate could be recommended. In this document, we note that PMMoV may show promise but is at present not ready for online monitoring and as yet has not been validated against pathogen removal in MBR.

The Tier 3 approach in WaterVal recommended undertaking a specific investigation to demonstrate the correlation between one or more online parameter(s) able to be continuously

monitored and the MBR pathogen removal performance. This investigation could be based on artificially varying the system removal performance (if normal variation was not significant enough to form correlations), while simultaneously measuring an online water quality parameter and carrying out challenge testing as per the method described in WaterVal Tier 2 (WaterSecure, 2017).

In addition to the requirement for continuous online monitoring that is correlated with LRV, WaterVal required the MBR to be operated at all times within an operational envelope. The operational envelope was defined by the limits of significant influencing factors. The influencing factors could be feedwater quality parameters and or operational variables which were shown to significantly influence LRV during the validation study.

Due to the expense and rarity of pathogen analysis laboratories in Australia, Tier 2 validation in WaterVal only necessitated analysis of indicator microorganisms (*C. perfringens* for protozoa, *E. coli* for bacteria and male-specific and somatic coliphages for viruses).

Unless there are significant improvements in the resolution of monitoring techniques provided by current surrogates, Tier 3 is not expected to verify higher LRVs than Tier 2 and it is likely that LRVs will be equivalent.

2.5.2 Turbidity as a Tier 3 Example from WaterVal

In order for turbidity to correlate with LRV, the assumption was made that LRV of suspended solids is equal to LRV of pathogens across the membrane. This assumption was verified at bench scale with fiber-cutting studies for *C. perfringens*, somatic coliphages, and *E. coli* (Branch, 2016). The following example was proposed in WaterVal as a method to correlate turbidity for monitoring of LRV (WaterSecure, 2017).

- Step 1: A linear correlation of turbidity and suspended solids should be determined. It is recommended that systematic spiking of activated sludge into MBR permeate be conducted, the solutions circulated at design flow through the commercial turbidity meter to be used, the turbidity recorded, and suspended solids analyzed for each solution. It is recommended that at least 6 different turbidities are analyzed, covering the proposed operating range of the instrument under field conditions (i.e., 0.1 - 1.5 NTU).). Following this approach, previous correlations have been found where a conversion factor can be made. For example: TSS(mg/L) = (1.3 to 4.3) x Turbidity (NTU) (Branch, 2016).
- Step 2: The conversion factor (in this example 4.32 [(mg/L of TSS)/NTU]) can then be used to calculate LRV across the membrane as per Equation 2-9.

$$LRV = LRV_{Bio} + \log_{10}\left[\frac{MLSS}{4.32 \times Filtrate\ Turbidity}\right] \qquad \text{(Equation 2-9)}$$

- Step 3: Provided MLSS is measured regularly or by an online instrument, LRV_{SS} can represent the removal across the membrane. LRV_{Bio} must still be accounted for and is pathogen and operating condition specific. In the WaterVal document, conservative default values were proposed below, based on the 5th percentile values obtained from the extensive site visit data collected as part of NatVal (Branch and Le-Clech, 2015). Note that negative LRVs are possible due to slow biological degradation and accumulation of organisms within the reactor. The values chosen for use in the indicative model are shown in Table 2-3 below.

Table 2-4. LRV_{Bio} Values from the NatVal Site Visits.
(Data from Branch and Le-Clech 2015)

Indicator Microorganism	LRV_{Bio} Probability Density Function Parameters			
	Mean	Standard Deviation	5th Percentile	95th Percentile
C. perfringens	-1.4	0.4	-2.0	-0.7
E. coli	0.9	0.7	-0.2	2.0
Somatic coliphages	0.0	1.0	-1.7	1.6
Male specific coliphages	1.1	1.0	-0.7	2.8

Using the default LRV_{Bio} values, LRV_{MBR} can then be calculated as a continuous function for a given MLSS.

An example was included in WaterVal using a conversion factor of 4.3 mg.L-1.NTU-1, MLSS of 5000 mg/L-1, and microorganism specific 5th percentile values for LRV_{Bio} from Table 2-3 and is reproduced below in Figure 2-6.

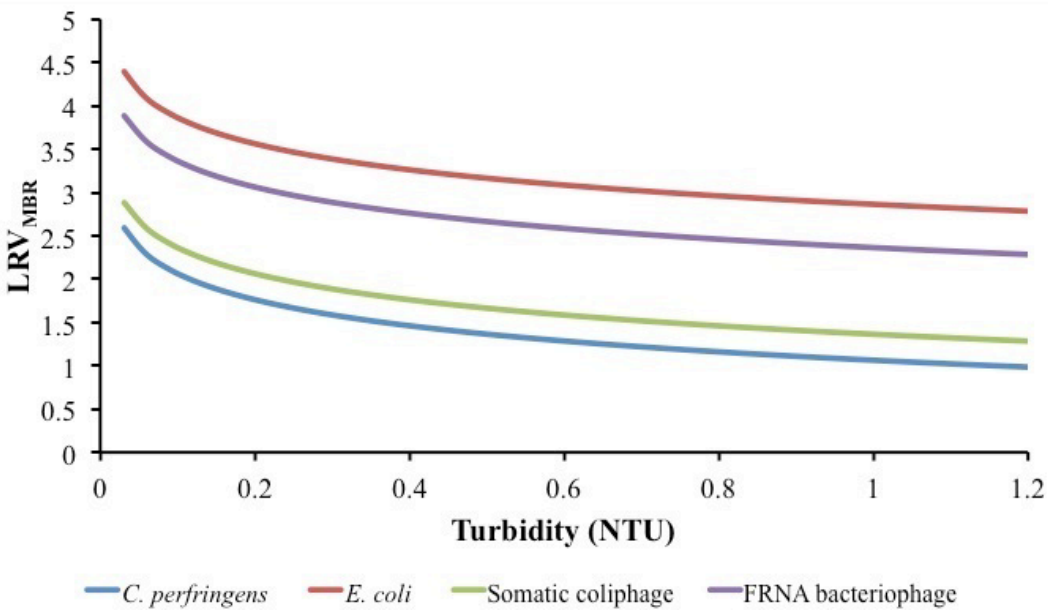


Figure 2-6. LRV_{MBR}, Predicted with Turbidity, for *C. perfringens*, *E. coli*, Male-Specific and Somatic Coliphages at an MLSS of 5000 mg.
L^{-1} and using 5th percentile LRV_{Bio} from Table 8 - 3, data from WaterSecure 2017.

- Step 4: The LRV_{MBR} model is conservative due to the LRV_{Bio} values adopted. To achieve higher LRVs, determination of site specific LRV_{Bio} could be conducted. If LRV_{Bio} is to be determined, the same sampling regime specified in Tier 2 should be conducted, with additional testing of activated sludge concentrations. Ideally, an online monitoring technique could be correlated with LRV_{Bio} and a LRV_{MBR} calculated continuously by measuring both LRV_{Bio} and turbidity. However, at this time, no such technique is known, although TOC removal monitoring was shown to have some promise.

A turbidity critical control limit (CCL) can then be selected and corresponding LRVs established.

Figure 2-6 highlights the more important contributor to this model, which is LRV_{Bio} value (Table 2-3). While all the indicators in Figure 2-6 follow an identical trend, LRV_{Bio} value caused significant deviations of up to 2 log, as in Equation 2-9, LRV_{Bio} is directly proportional to LRV_{MBR}. Another interesting trend identified by the model was the limited impact of increasing turbidity beyond 0.2 NTU, which is commonly used as a CCL in MBRs. The minimal change of LRV with a turbidity increase from 0.2 to 1 NTU is due to the logarithmic relationship linking the parameters (i.e., a significant change in LRV requires an order of magnitude change in turbidity).

The WaterVal document recommended that sampling should take place in a range a measurable turbidity change that can be expected and assessing historical turbidity data can assist in ascertaining a baseline. To ensure the LRVs identified are conservative, the sampling program and turbidity correlation should be performed under the most conservative conditions and therefore the lowest MLSS concentration and highest flux within the operating envelope.

When the model above was evaluated at bench scale, an excellent correlation of LRV_{Mem} and LRV_{SS} was observed for *E. coli* and *C. perfringens* in filtrate samples, where it was possible to detect both MLSS and indicator microorganism (turbidity > 0.19 NTU). The conservative, generic model illustrated in Figure 2-6 was developed to allow calculation LRV_{MBR} based on a given operating MLSS and instantaneous permeate turbidity. Results from the model imply that at a CCL of 0.2 NTU, turbidity should be capable of assuring and overall LRV of 1.5 to 3.5 depending on the removal of a particular microorganism in the activated sludge prior to reaching the membrane (i.e., the value of LRV_{Bio}).

The overall LRV proposed by the model was subject to large variations caused by a generic LRV_{Bio} determined across multiple sites and operating conditions. The calculated parameter, Log_{10} (MLSS/turbidity), was used to evaluate the online model for suspended solids removal across the membrane. Unfortunately, when tested on the entire NatVal data set, the only statistically significant correlations were observed for *E. coli,* total coliforms and somatic coliphage removal. It is possible that the lower frequency of detection of *C. perfringens* and male-specific coliphages may have hindered this correlation. Also, the correlation coefficients were low, indicating a weak relationship, and there may have been too much site-to-site variation for the model to function globally (Branch, 2016). On a single site, with more stable operating conditions favorable to LRV_{Bio} (i.e., long HRT) a greater LRV_{MBR} could be demonstrated. Ideally, future work would arrive at a monitoring strategy for the activated sludge that could be correlated with LRV_{Bio} and allow real time calculation of overall LRV.

2.5.3 Gap Analysis of WaterVal Tier 3 Approach

In general, the WaterVal approach shows promise. However, there are limitations and opportunities for improvement. The following areas of the WaterVal approach should be assessed:

- Pathogens, indicators and analytical recovery:
  - Predominantly, the work in WaterVal included analysis of indicator organisms only. Although positive controls were run, there was no attempt to correct indicator analysis results for recovery.
  - More importantly, there is a need for more pathogen data, and it is recommended that Tier 3 validation be conducted such that the correlation is shown to be valid for the target pathogens *Cryptosporidium* and enterovirus, named in the Tier 2 validation protocol (Salveson et al., 2021). Ideally, a correlation would also be developed for the indicator microorganisms and the target pathogens.
  - When considering incorporation of pathogen monitoring for Tier 3 correlation development, the following challenges are noted:
    - Analytical recovery - The analytical recovery is variable, and it is recommended to perform additional matrix spike analysis to ensure that all data collected can be corrected for recovery.
    - Matrix spikes to determine the recovery of viruses more than double analysis costs which are typically more than $1000 per sample. The necessary frequency of virus matrix spikes for enterovirus should be carefully considered.
    - Analysis Turn Around Time - There are a range of analysis turnaround times from conventional laboratories, from 1 - 2 weeks for viruses (by qPCR) and *Cryptosporidium* to up to 8 weeks or more for viruses by cell culture. The extended turnaround time would need to be considered in the commissioning schedule if validation was a condition for a system operating permit.
- Membrane configuration and operating parameters:
  - The WaterVal project collected data from submerged hollow fiber and flat sheet systems. A majority of which were hollow fiber. Data is sparse for crossflow MBR systems as these systems were not readily available for study.
- Turbidity correlates well with suspended solids removal and is already included as a monitoring surrogate. There is uncertainty on how well turbidity will correlate with LRV, subject to the following factors:
  - Should turbidity be used alone, or used to calculate an LRV_{Mem} of suspended solids?
  - Is the breakthrough of suspended solids proportional to pathogen breakthrough? Results from the NatVal study suggest that under compromised membrane integrity, LRV_{Mem} of indicators and suspended solids was equivalent. It is not yet certain if turbidity breakthrough will be equivalent to LRV for defects smaller than a broken fiber.
- PDT was not included in the WaterVal MBR project. However, given its inclusion as an option for augmented monitoring in Tier 2 and potential as a conservative and selective surrogate for membrane removal, its evaluation should be included in U.S. Tier 3 validation plans. However, it is recommended to explore a hybrid application which does not strictly follow guidance in the USEPA MFGM in the selection of a test pressure (i.e., looking a lower

test pressures) or for calculation of a VCF, as this will severely limit the ability of PDT to demonstrate meaningful LRVs. Rather, it is suggested that a pseudo VCF could be informed by system specific sampling of the activated sludge and determination of a bioreactor removal relevant to the site.

- Also, TOC monitoring was shown to have potential for bioreactor removal assurance, but more data are needed across a range of sites. Correlations with pathogen removal should also be assessed.
- Systematic experiments across a range of sites to determine kinetic degradation coefficients for pathogens in the bioreactor could provide meaningful information to support bioreactor validation models. However, to date, the data available are limited.

CHAPTER 3

Utility and Supplier Survey

MBRs provide substantial value to potable water reuse projects, due to the footprint, process intensification, cost of implementation, and water quality as a feed for subsequent purification by an Advanced Water Purification Facility (AWPF). A handful of progressive utilities have implemented, or are implementing, potable water reuse programs based upon the core technology of MBR, including small systems (~1 million gallons per day [mgd]) and large systems (>100 mgd). Further, select MBR suppliers are dedicating time and effort to better understand MBR pathogen removal. As part of this grant, a small list of utilities and suppliers were contacted to understand:

- The value of a Tier 1, Tier 2, and Tier 3 Validation to Potable Reuse;
- Existing and future operational conditions for potable reuse MBRs; and
- Challenges to monitoring and operations and maintenance that may result from the use of different Tiers.

Tables 3-1 and 3-2 below list the contacted utilities and suppliers, respectively. For each group, a short presentation of Tier 1, Tier 2, and Tier 3 efforts was presented, followed by a discussion of group specific surveys (shown below).

The sections below include the surveys as delivered to utilities and suppliers.

Table 3-5. Utility Outreach.

Facility Name	Project Status	Capacity, mgd	AWPF Train
Rio Rancho, New Mexico	Operational.	~1 mgd average flow, ~1.9 mgd peak flow.	MBR, O_3, BAC
Morro Bay, California	Under construction.	~1 mgd average flow, ~1.9 mgd peak flow.	MBR, RO, UVAOP
Metropolitan Water District, California	Demonstration is operational. Full-scale is in planning phase.	1 mgd AWPF, demonstration scale for future >100 mgd project.	MBR, RO, UVAOP
City of Los Angeles, California	Demonstration is under construction. Full-scale is in planning phase.	1 mgd AWPF, demonstration scale for future >100 mgd project.	MBR, RO, UVAOP
South Jordan (via Jordan Basin WRF)	Full-scale is operational. Demonstration is under design.	The Jordan Basin WRF MBR has a capacity 13.9 mgd average and 27 mgd peak flow.	Existing MBR, demonstration facility with MBR, O_3, BAC, GAC, UV under design.
Abbreviations: BAC - Biologically Active Carbon Filtration; O_3 - Ozone; UVAOP - Ultraviolet Advanced Oxidation Process.			

Table 3-6. Supplier Outreach.

Supplier	Product Offering	Notes
DuPont	Hollow fiber UF MBR	Development of PDT MBR membrane monitoring system, including the use of PDT at Gippsland Australia. Undergoing extensive product validation with the Metropolitan Water District at the Carson Joint Water Pollution Control Plant (CA). Will undergo Tier 2 testing at the City of Los Angeles (Hyperion WRP) pure water demonstration facility (2022). Installations Studied as part of (SCVWD, 2017)
Fibracast	Hollow fiber UF MBR in membrane sheets	Limited pathogen removal testing at several locations.
Koch	Hollow fiber ultrafiltration MBR	Will undergo Tier 2 testing at the City of Los Angeles (Hyperion WRP) pure water demonstration facility (2022).
Kubota	Flat sheet MF MBR	Undergoing extensive Tier 2 product validation at a California Utility
Memstar	Hollow fiber UF MBR	No available validation data but installations operating in Singapore and China.
Suez		Undergoing extensive product validation with the Metropolitan Water District at the Carson Joint Water Pollution Control Plant (CA). Will undergo Tier 2 testing at the City of Los Angeles (Hyperion WRP) pure water demonstration facility (2022). Installations Studied as part of (SCVWD, 2017). Long term operation of an IPR scheme at Hamby WRF Abilene, TX.
Note: The survey is not an endorsement of MBR product vendors but a description of the survey participants.		

3.1 Utility Survey

Each utility and treatment train relies upon MBR for different levels of credited pathogen removal; with the required level of credit dependent upon the unit processes in the overall Advanced Water Treatment Facility (AWTF) and any attenuation in the environment, all of which will be system-specific and related to the regulatory structure or program objectives. For example, the MBR LRV may not be as critical for an AWTF with additional redundancy provided by redundant treatment systems or that recharge groundwater basins with very long groundwater travel times (e.g., > 6 months). However, the MBR LRV may be critical for some purification systems with fewer treatment barriers, shorter aquifer storage/travel times, or particularly for DPR systems in which added safety margins are desired. Typically, the key focus is upon protozoa removal instead of virus removal and the need to gain a better understanding of MBR system performance and surrogate monitoring. To better understand utility concerns about their existing or future MBR for potable reuse, the following survey was developed, with a goal of using the information to focus this Tier 3 investigation. This survey focuses first upon operational conditions, operations and maintenance challenges and efforts, and existing monitoring requirements, then shifts to better understand the value of Tier 1, Tier 2, and Tier 3 validation efforts (Figure 3-1).

3.2 Supplier Survey

Equipment suppliers are an equally important partner in this work, as concepts must be ***implementable*** and of ***reasonable cost*** for them to flourish in the marketplace. Suppliers have different approaches to membrane and process monitoring and different visions of how MBR

should be part of potable reuse systems. To that end, the goal of the survey was to better understand what design conditions, operational envelopes, and treatment and monitoring system innovations are available in the present and future for these suppliers (Figure 3-2).

Utility Survey

Your input is needed to best understand what role and level of significance your MBR plays in your potable reuse program, including both water quality benefits and operations and maintenance efforts and cost. Your responses to the following questions will help this project team best design a Tier 3 protocol for MBR pathogen removal.

Question	Answers
MBR Supplier?	
Average and Peak Flow?	
What are your operational conditions, **see supplemental table below**. This data is critical to expand the "Operational Envelope" for validated MBR systems.	
History of Compliance Challenges (e.g., turbidity)?	
Frequency and Level of Effort for Membrane Maintenance and Monitoring System Maintenance? Qualitative and quantitative details needed.	
Online Monitoring Systems and Calibration Frequency and Success?	
Grab Sample Monitoring (e.g., pathogens, surrogates)? Representative results?	
Membrane Damage Events (causes, detections, response)?	
Regulatory Challenges Related to MBR?	
Water Quality Challenges Related to MBR (surrogates, pathogens, other)?	
Operational Concerns for MBR (fouling, flux, repairs)?	
What technological advance(s) is (are) needed for you to have greater confidence in MBR performance?	
What technological advance(s) is (are) needed for a lower O&M burden?	
What relative benefit would you get from Tier 1, Tier 2, or Tier 3 validation? **See supplemental table below**.	

Parameter	Minimum Value	Maximum Value
Bioreactor pH		
Bioreactor Dissolved Oxygen, mg/L		
Bioreactor Temperature, °C		
Solids Retention Time, days		

Figure 3-7. Utility Survey.

Supplier Survey

Your input is needed to best understand your vision of MBRs for potable water reuse, both now and in the future. Your responses to the following questions will help this project team best design a Tier 3 protocol for MBR pathogen removal.

Question	Answers
What design and operational envelope do you see for your systems? **See supplemental table below**. This data is critical to expand the "Operational Envelope" for validated MBR systems.	
What product challenges exist to implement MBR for potable reuse?	
What monitoring system challenges exist to implement MBR for potable reuse?	
What regulatory hurdles does your company face when "selling" MBR for potable reuse?	
How do the Tier 1, Tier 2, and Tier 3 efforts help, or hinder, MBR application for potable reuse?	
What future potable reuse projects are you targeting and how could a Tier 3 validation support your success?	
What product changes are you implementing to better compete for potable reuse projects?	
What key recommendations would you suggest are part of the Tier 3 Protocol?	

Parameter	Minimum Value	Maximum Value
Bioreactor pH		
Bioreactor Dissolved Oxygen, mg/L		
Bioreactor Temperature, °C		
Solids Retention Time, days		
Hydraulic Retention Time, hours		
Mixed Liquor Suspended Solids, g/L		
Transmembrane Pressure, kPa [psi]		
Flux, L/m²/h [gfd]		
Turbidity, NTU		

Figure 3-8. Supplier Survey Utility Survey Results.

3.2.1 Utility Survey Common Findings

Common issues/conclusions that were noted by more than one Utility Survey are summarized below.

3.2.1.1 The Value of Real Time Monitoring

Guidance for potable water reuse target pathogen treatment sufficient to minimize the annual risk of infection to 1 x10^{-4} (1 in 10,000 per person per year) (NWRI, 2016, 2015; SWRCB, 2019; Trussell et al., 2013). Within California, the current guidance for DPR is to minimize risk based upon daily exposure (SWRCB, 2019), which results in a risk of infection target of 2.7 x 10^{-7} infections per day, essentially requiring a greater focus on daily LRV verification and real-time monitoring to ensure that the daily risk of infection is maintained at less than 1/365th of the

annual. An alternative would be to increase the overall treatment capacity by 2.6 LRV (i.e., log10[365]) as a means to ensure that there is additional buffer such that the likelihood of exceeding the daily target is significantly reduced.

The Tier 3 approach, if proven successful, would accomplish the goal related to assuring that daily LRV requirements in DPR are continuously verified with real time or near real time monitoring ability.

3.2.1.2 Complexity and Cost

Tier 1 credits come with very low complexity and cost. For utilities that do not need higher credits, Tier 1 is the preferred approach. If additional LRV credits are needed, then a Tier 2 or Tier 3 approach is needed, with Tier 3 potentially providing more value for a DPR project because of the ability to monitor performance in real time, and thus provide greater confidence in Risk (see above).

3.2.1.3 The Value of Higher LRV

For IPR systems that utilize a groundwater basin or a surface water body and have extended time in the environment (e.g., >6 months), some pathogen reduction can conservatively be assigned. As an example, California regulations assign 1-log reduction of virus per month of time in the environment, associated entirely with die-off of the virus (SWRCB, 2018). For IPR projects that store the water in the environment or extended periods of time, such as 6 months, the Tier 1 LRV for virus of 1.0 is more than sufficient credit as part of a robust treatment train. However, for limited time in the environment, such as 2 months or less, obtaining higher LRV for virus via Tier 2 or Tier 3 will be valuable. Some of the participating utilities specifically commented that Tier 1 was sufficient for IPR, and that Tier 2 or Tier 3 is needed for DPR. Further, comments from participating utilities suggest that Tier 3 is ONLY important for DPR and would not see application in IPR projects unless it utilized an inexpensive and reliable monitoring system.

3.2.1.4 Operations and Maintenance Staff Impacts

As listed above, Tier 1 credits come with minimal impacts to operations and maintenance staff, essentially equivalent level of effort to typical MBR operations. Tier 2 credits will require increased staff time pertaining to: (1) instrument technician time working with increased online or periodic online monitoring and (2) extensive sampling efforts. Tier 3 credits may reduce the sampling efforts compared to Tier 2 credits but will increase the level of effort and the significance of the effort of the instrument technician that is working with online monitoring systems.

For Tier 3 to be useful and provide value beyond Tier 2, the system must: (1) correlate the surrogate to pathogen removal, (2) have confidence in the accuracy and precision and sensitivity of the monitoring system, and (3) be robust and easy to maintain. Failure in any of these items, including maintenance challenges, will reduce or eliminate the benefit of Tier 3 systems.

3.2.2 Specific Utility Survey Findings

The view of the MBR end user is presented here, with direct feedback from 5 different utilities/municipalities that are implementing, or have implemented, MBR based potable water reuse projects. These perspectives reflect both large and small systems, located across the United States, and using different combinations of treatment technologies to make new water from wastewater effluent. The interview responses are presented within 7 categories of discussion.

3.2.2.1 Tier 1 Credits

A Tier 1 system includes a virus credit of 1 LRV and a protozoa credit of 2.5 LRV. The monitoring includes maintaining a turbidity <0.2 NTU 95% of the time with a not to exceed value of 0.5 NTU. A Tier 1 system may include monitoring of a secondary surrogate, such as PDT or total coliform on a daily or frequent basis (Salveson et al., 2021).

O&M Requirements

The operations and maintenance (O&M) efforts associated with Tier 1 are equal to, or only marginally greater than, a conventional MBR O&M program. Such a simple program has significant value to utilities. Example O&M efforts include calibration of turbidity meters and may include periodic PDT or total coliform testing. Membrane repair, such as pinning, does not normally occur with MBR systems, and would not be anticipated to be needed for a Tier 1 operation. It is anticipated that Tier 1 LRVs are conservative enough that they should be achieved throughout the membrane product life.

Direct Benefits

There are several direct benefits of a Tier 1 system, including:

- Open to all MBR suppliers, allowing for a competitive marketplace.
- No pathogen sampling is required to confirm performance, reducing operational cost and expertise requirement.
- Flexible operation, not requiring the MBR to operate within a restrictive “validation window” applied in the WaterVal guidelines, with the exception of turbidity requirements.
- Conservative LRV assignment, which allows for regulatory confidence.

Challenges

There are several direct challenges of a Tier 1 system, including:

- LRV credits may be on the margin for some systems. As an example, an MBR, RO, UV AOP system in the State of California, under the Tier 1 system, would:
  - Receive 8.5 LRV of virus (1 - MBR, 1.5 - RO, 6 - UV) without on-site proof of performance.
  - Receive 10.0 LRV of protozoa (2.5 - MBR, 1.5 - RO, 6 - UV) without on-site proof of performance.
  - Require an additional 3.5 LRV of virus and just marginally reach the LRV of protozoa to attain the 12/10/10 (virus/*Giardia*/*Cryptosporidium*) LRV requirements of the State of California.

- Additional LRV credits can be obtained by adding treatment and/or monitoring systems to supplement a Tier 1 system, including:
  - Increasing virus LRV credits by adding retention time (e.g., in a groundwater basin), adding engineering treatment (e.g., free chlorination) or improving monitoring systems (e.g., sampling for novel RO performance surrogates such as strontium or sulfate across RO to increase RO credits).
  - Increasing protozoa LRV credits by adding engineering treatment (e.g., additional filtration/separation barriers) or improving monitoring systems (e.g., sampling for novel RO performance surrogates such as strontium or sulfate across RO to increase RO credits).

Implementation Issues
A program based upon a Tier 1 system is simple and presents the least implementation issues from a treatment, monitoring, and O&M standpoint.

The Tier 1 approach also provides an important demarcation line between the WWTP and the AWPF, which is a common institutional challenge when the WWTP and the AWPF are owned and run by separate entities. A Tier 1 approach allows for the WWTP to continue "business as normal" and not become intertwined in a more costly and complex monitoring, sampling, and O&M program to maintain a greater LRV credit.

Other Discussion Points
Overall, the Tier 1 system was identified as preferable for IPR systems and for small communities that do not wish to be on the front edge of technology development.

3.2.2.2 Tier 2 Credits
A Tier 2 system, based upon ongoing Tier 2 validation testing of an older MF equipped MBR, is anticipated to result in a virus credit (based on the conservative 5^{th} percentile) of > 2 LRV and a protozoa credit of > 3.5 LRV (Fontaine and Morris, 2020). Monitoring would include the Tier 1 turbidity criteria plus the need for a secondary surrogate, such as PDT and/or total coliform monitoring. In addition, the Salveson et al., 2021 Tier 2 protocol recommends that there would be MBR product and site-specific testing phases that require sampling for indicator microorganisms and pathogens.

O&M Requirements
The MBR O&M efforts associated with Tier 2 is expected to be marginally greater compared to Tier 1 systems. It is anticipated that the increased monitoring to justify higher LRVs may recognize subtle membrane integrity failures and require membrane repairs in order to continually maintain higher LRV credits. In addition, a substantial operational budget will need to be set aside to cover ongoing indicator microorganism and pathogen sampling requirements.

Direct Benefits
There are several direct benefits of a Tier 2 system, including:

- Provides measurably greater LRVs than Tier 1, which is perceived as more valuable for DPR projects.

- May allow for less treatment processes to meet regulated pathogen removal targets.
- Allows for shorter groundwater travel time for groundwater recharge potable reuse projects.
- Can result in pathogen LRV credits in excess of regulatory requirements, creating a buffer that allows for greater operational flexibility of the entire treatment train.
- Provides greater confidence in MBR performance by staff and regulatory personnel.

Challenges
There are several direct challenges of a Tier 2 system, including:

- Higher LRVs may be supplier specific, limiting competition.
- Operation may be confined within the tested range of performance for the tested MBR system.
- Performance must be proven at full-scale with continued sampling at frequencies between twice per month to quarterly, conditional on performance, over the life of the installation.
- Degradation in performance over time presents an economic risk to the MBR owner. Economic risks may include need for earlier membrane replacement, additional maintenance and/or additional sampling and testing.
- Site-specific testing is costly, for both pathogens and surrogates.
- Implementing a secondary surrogate adds both cost and potentially instrumentation.

Implementation Issues
Implementing a program based upon a Tier 2 system requires greater investment of engineering, laboratory, and instrumentation over the life of the project. With sufficient data generation, some of these burdens may be reduced over time.

Other Discussion Points
Overall, the Tier 2 system was identified as preferable for DPR systems or for utilities that have the financial and staff flexibility to invest in higher LRVs. These higher LRVs will provide greater confidence in water quality and greater flexibility in operations (e.g., sufficient operational flexibility to overcome potential off-spec performance by other treatment barriers).

The immediate need/reliance on the purified water by a community also impacts the value of a Tier 2 system. For some communities, where an AWPF can go offline to fix monitoring or treatment items, the added buffer of higher LRVs is not significantly important. For a system needing greater reliability of production, higher LRVs could be very important as a means to provide a continual source of water that is protective of public health.

3.2.2.3 Tier 3 Credits
A Tier 3 system is anticipated to provide similar pathogen LRV credits to a Tier 2 system but do so with increased monitoring and thus provide greater confidence in performance in real time.

O&M Requirements
The O&M efforts associated with Tier 3 is expected to be measurably greater compared to Tier 1 and Tier 2 systems, requiring some undetermined level of membrane repairs and enhanced monitoring systems. However, it is anticipated that a lower ongoing analytical cost

will be required for pathogen analysis, as the pathogen verification function is achieved by correlated online monitoring systems.

Direct Benefits
There are several direct benefits of a Tier 3 system, including:

- Provides measurably greater LRVs than Tier 1 but similar to Tier 2.
- The higher LRV provides greater benefit to DPR projects.
- Direct correlation between a monitoring result and pathogen LRV, in real time, provides a significant benefit to DPR projects.
- Dramatically reduced response time, the time between a potential off-spec event and a response, provides a direct benefit to potable reuse risk calculations.
- May allow for reduced pathogen and surrogate monitoring requirements and costs.
- Like Tier 2, provides an opportunity for less treatment processes, shorter groundwater travel time (for IPR projects), less treatment processes to meet pathogen reduction goals, greater operational flexibility of the entire treatment train.
- Provides greater confidence in MBR performance by staff and regulatory personnel than Tier 1 and Tier 2 systems.

Challenges
There are several direct challenges of a Tier 3 system, including:

- Higher LRVs may be equipment and monitoring system specific, potentially limiting application to Suppliers and Users with substantial resources to perform the validation.
- Operation may be confined within the tested range of performance for the tested MBR system.
- Site-specific testing is costly, for both pathogens and surrogates, though the Tier 3 monitoring system should result in less cost compared to Tier 2.
- As of today, turbidity within the regulated range has yet to be proven sufficiently sensitive for use as a Tier 3 monitoring system.
- Other monitoring systems, such as PDT and TSS, are also as of yet unproven for Tier 3 monitoring.
- If a Tier 3 system is used, and the full LRVs are necessary for public health protection (i.e., LRVs are on margin) and if monitoring systems fail, diversion of water is required.

Implementation Issues
Like Tier 2, implementing a program based upon a Tier 3 system presents requires greater investment of engineering, laboratory, and instrumentation over the life of the project. With sufficient data, the laboratory costs are anticipated to be reduced relative to Tier 2, but the long-term instrumentation verification and maintenance are expected to be significant.

Other Discussion Points
Overall, the Tier 3 system is seen as much more valuable and relevant for DPR systems. With the unproven nature of Tier 3 monitoring systems, utilities are wary of the cost and benefit of such systems. For Tier 3 system success the industry must: (1) Find a correlation, (2) have

available technology with acceptable accuracy and precision and sensitivity, and (3) be robust and easy to maintain. Unfortunately, no presently available technology has been shown to satisfy all three of these criteria. In particular, pathogen correlation data even with turbidity monitoring approaches is not available.

3.2.3 Supplier Surveys

A summary and synthesis of results is presented below to capture the perspectives of MBR suppliers.

The following perspectives were summarized from available supplier surveys.

3.2.3.1 Cost

Pathogen analysis is costly and a hindrance. A number of suppliers were concerned that the potable reuse market for Tier 2 validation was not large enough to justify costs. Projects specifically requiring the efforts would need to carry the cost.

3.2.3.2 Monitoring limitations

Certain configurations of membranes and membrane products are not suitable for particular types of monitoring, e.g., PDT. A greater diversity of monitoring techniques that are either independent of MBR membrane configuration or cover a wide variety of configurations would benefit end users allow choice between vendors. Certain monitoring techniques are unable to account for particular removal mechanisms. None of the commonly applied monitoring criteria could identify source water upsets that could reduce bioreactor treatment efficacy.

There were concerns that specifying additional monitoring, other than turbidity, may present implementation challenges. Air bubbles are a common interference with turbidity monitoring and may thus falsely imply performance failure.

Measurement of pathogens is a significant limitation given the site-to-site variability that can result in frequent non-detects that make it difficult to demonstrate robust performance and correlation with monitoring techniques. Pre-validation test sites that have high pathogen loads may benefit certain suppliers by allowing demonstration of higher removal.

3.2.3.3 LRV Credit

Tier 1 and Tier 2 LRVs are already sufficient for most potable reuse applications. Most membrane suppliers would still pursue Tier 2 to demonstrate product validity and accept that it may provide a competitive edge. The full range of removal mechanisms should be considered as part of validation, not just the membrane integrity.

3.2.3.4 Regulatory Hurdles

There are no universal well-defined criteria, and this makes it difficult for suppliers to meet the needs of all customers. It can be a challenge for suppliers when permitting or validation activities accepted in one country, or even one state within a country, are not able to be transferred to other jurisdictions. Advanced acceptance of the protocols, such as a letter from the regulatory bodies, would reduce uncertainty and help utilities move forward with designs.

3.2.3.5 Ongoing Improvement and Development of Validation Protocols

Suppliers supported that the requirements of validation protocols must be reviewed and updated as new information becomes available. This was noted with particular reference to potentially being able to justify higher Tier 1 LRVs that were perhaps supplier specific after a significant body of Tier 2 evidence had been gathered.

3.2.4 MBR Design and Operational Range

With the early precedent set by WaterSecure (2017) for Tier 1 credit validation range, the engineering community has focused upon that range and raised concerns about the validity of operating outside of that range. Salveson et al., 2021 provided a contrary position, clearly allocating Tier 1 pathogen credits independent of operational parameters as long as turbidity criteria is met. The information below presents the operational ranges for MBR systems, both large and small and compares that range with (WaterSecure 2017). This information is compared in the tables below with the "Tier 1" Australian Validation Range (Table 3-3), which is the range of operation that was allowed if the Australian Tier 1 credits were to be granted.

Of note, most of the U.S. facilities and suppliers specify operational parameters outside the range that was observed and specified as an operational envelope during the WaterVal research project. As noted in supplier survey responses, any Tier 3 system, would ideally be able to perform appropriately within the normal specified range of acceptable operation and the current WaterVal range is too narrow. The acceptable range of operating criteria based on survey of utilities and suppliers is summarized in Table 3-4 and 3-5. Ideally, any proposed monitoring approach would be valid at least across the entire range of operational conditions proposed.

Table 3-7. MBR Operating Envelope for Adoption of Conservative Tier 1 LRV from WaterVal (WaterSecure, 2017).
Table also shows the actual values of the 5th and 95th percentile in brackets noted during the WaterVal sampling campaign (Data from Branch and Le-Clech, 2015).

Parameter	Minimum Value[4]	Maximum Value	Correlation with LRV[1]
Bioreactor pH	(5.5) 6	(7.5) 8	+ve
Bioreactor Dissolved Oxygen, mg/L[2]	(0.5) 1	7 (7.5)	-ve
Bioreactor Temperature, °C	16	(29) 30	+ve
Solids Retention Time, days	11	- (147)	+ve
Hydraulic Retention Time, hours	6	- (45)	+ve
Mixed Liquor Suspended Solids, g/L	3[5]	- (14)	-ve
Transmembrane Pressure, kPa (psi)	3[0.4][5]	- (47) [6.8]	-ve
Flux, $L/m^2/h$ (gfd)	-	30 [17.7]	-ve
Turbidity, NTU	-	0.25 (0.4)[3]	-ve

Notes:

1. +ve means as parameter increases, LRV typically higher, -ve means as parameter increases LRV typically lower.
2. While a high dissolved oxygen was observed to occur with low LRV there may have been sampling bias issues. There is no theoretical basis to why a high DO should cause a low LRV.
3. Reduced to 0.2 from original 95th percentile limits of 0.3 NTU for membranes with pore size < 0.1 micron and 0.4 NTU for the entire set of data investigated.
4. Values in bold represent limits beyond which a reduction in Tier 1 LRVs was concluded to be likely.
5. Lower limit for MLSS included due to causal suspicion that operation without biomass would lead to poor LRV, even if not demonstrated. Lower limit for TMP included as high permeability (that is low TMP and high flux) was found to correlate with low LRV.

Abbreviations: gfd - gallons per square foot per day.

Table 3-8. Supplier Operational Range Comparative Table.

Parameter	WaterVal Range		Suez		Kubota		DuPont		Fibracast		KOCH		Memstar	
	Min Value[3]	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value
Bioreactor pH	(5.5) 6	(7.5) 8	5	9	6	9	6	9	6	9	6.5	8.0	Biology dependent	
Bioreactor Dissolved Oxygen, mg/L	(0.5) 1	7 (7.5)	>1 mg/L in aerobic zone	not specified	0.7	5	0	8	0.5	no maximum	0.5	4.0	2	-
Bioreactor Temperature, °C	16	(29) 30	5	40	6	40	1	40	1	40	5	35	5	40
Solids Retention Time, days [4]	11 [4]	147 [4]	8/8 (aerobic/total)	30+ (aerobic/total)	6/10 (aerobic/total)	no maximum	8[2] (total)	50[2] (total)	>20 (aerobic/ total)	<60 (aerobic/ total)	8[7]	40[7]	Feedwater dependent	
Hydraulic Retention Time, hours [5][6]	6	45	3/3 (bioreactor/ total)	24/50+ (bioreactor/ total)	1.2/4 (membrane tank/total)	4/10+ (membrane tank/total)	NA	NA	4 (bioreactor/ total)	<15 (bioreactor/ total)	4	20	Feedwater dependent	
Mixed Liquor Suspended Solids, mg/L	3,000	14,000	No minimum	15,000	5,000	20,000	1,000	14,000	No minimum	15,000	4	12	4	12
Transmembrane Pressure, psi	0.4	6.8	0	8	0.05	3	-16	11	-0.1	-8	0.25	9	-	7
Flux, gfd	-	17.7	0	35	8.9/8 (instantaneous/ net)	40/36 (instantaneous/ net)	6	26.5	No minimum	30/25 (instantaneous/ net)	6/5 (instantaneous/ net)	30/27 (instantaneous/ net)	-	32/29 (instantaneous/ net)
Turbidity, NTU	-	0.2 (0.4)[1]	0.02	0.5	0	0.2	0	0.5	0.05	0.5	0.02	0.5	-	-

Notes:

1. Reduced to 0.2 from original 95th percentile limits of 0.3 NTU for membranes with pore size < 0.1 micron and 0.4 NTU for the entire set of data investigated.
2. SRT is determined based on design temperature. SRT is considered to be the minimum average value over any 30-day period, with SRT not exceeding 50 days. Low value shown is based upon T=20 degrees Celsius.
3. Values in "()" represent 5th and 95th percentile data from the research, whereas the values without brackets represent the values listed in Branch and Le-Clech, 2015.
4. Values shown are total SRT. Aerobic SRT is ~50% to 60% of total SRT.
5. Values shown are total HRT. Bioreactor HRT is ~90% of total HRT.
6. For Kubota, the membrane tank is part of the bioreactor tank.
7. Minimum SRT at Temperatures > 20 degrees Celsius, maximum SRT required at cold conditions, Temperatures < 10 degrees Celsius.

Table 3-9. User Operational Range Comparative Table.

Parameter	Australian Range		Rio Rancho New Mexico (Suez MBR)		Morro Bay California (Suez MBR)[2][6]		South Jordan Utah (Suez MBR)		Metropolitan Water District (Suez & DuPont MBRs)		City of Los Angeles (Suez, DuPont, Koch MBRs)[6]	
	Min Value[3]	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value	Min Value	Max Value
Bioreactor pH	(5.5) 6	(7.5) 8	7.3	7.7	6.7	8.5	7.09	8.83	6.5	7.5	6.5	7.5
Bioreactor Dissolved Oxygen, mg/L	(0.5) 1	7 (7.5)	2	3		<5	5.6	8.3	1.2	2.3	1	2.2
Bioreactor Temperature, °C	16	(29) 30	20	26	17	24	14.1	24.4	21	33	21	33
Solids Retention Time, days[4]	11[4]	147[4]	7/8 (aerobic/total)	10/12 (aerobic/total)	9/10.5 (aerobic/total)	10.5/14 (aerobic/total)	not provided	not provided	9/9 (aerobic/total)	9.5/9.5 (aerobic/total)	6/12 (aerobic/total)	7.2/12 (aerobic/total)
Hydraulic Retention Time, hours[5]	6	45	6/7 (bioreactor/total)	10/13 (bioreactor/total)	5.5/6 (bioreactor/total)	37/40 (bioreactor/total)	not provided	not provided	2.5/3.1 (bioreactor/total)	2.5/3.1 (bioreactor/total)	8/8.4 (bioreactor/total)	10.3/10.8 (bioreactor/total)
Mixed Liquor Suspended Solids, mg/L	3,000	14,000	6,000	10,000	5,500	7,500	not provided	not provided	1,000	1,300	4,500	9,000
Transmembrane Pressure, psi	0.4	6.8	-0.5	-5	-8	8		0.5	0.5	3	0.5	3
Flux, gfd	-	17.7	12	17	9 (net) 9.9 (instantaneous)	21.5 (net) 25.2 (instantaneous)	10	11	10	14	14	18
Turbidity, NTU	-	0.2 (0.4)[1]	0.07	5	0.01	0.2	0.01	0.81	0.02	<0.2	0.02	<0.2

Notes:

1. Reduced to 0.2 from original 95th percentile limits of 0.3 NTU for membranes with pore size < 0.1 micron and 0.4 NTU for the entire set of data investigated.
2. SRT = 8/1.029^(T-20), where SRT is the minimum average value over any 30-day period, with SRT not exceeding 50 days. Low value shown is based upon T=20 degrees Celsius.
3. Values in "()" represent 5th and 95th percentile data from the research, whereas the values without brackets represent the values listed in Branch and Le-Clech, 2015.
4. Values shown are total SRT. Aerobic SRT is ~50% to 60% of total SRT.
5. Values shown are total HRT. Bioreactor HRT is ~90% of total HRT.
6. Facility design parameters listed as they are not yet operational.

CHAPTER 4

Development of a Tier 3 Validation Protocol Outline

A goal of this research project is to develop the requirements for a Tier 3 MBR Validation protocol. A primary challenge of this work is the rapid and continued development of new research outside of this project. Some of that work has been done by project partners and is included herein to better guide a Tier 3 Validation Protocol Outline. This new work, presented here with the permission of the authors, shows promise that a single or combination of surrogates can be used to document a threshold or stepped LRV based upon measured parameters.

This section presents a summary of a brainstorming workshop followed by some of the latest research on Tier 3 and ends with a recommended outline for a Tier 3 Validation Protocol.

4.1 Workshop

An online workshop was hosted with the technical advisory committee (TAC) members and core members of the research team on the 29th of March 2021. The workshop consisted of an orientation presentation on the work to date, including the literature review and utility and supplier surveys, followed by a series of focus questions that were defined to scope potential requirements for a Tier 3 validation study and protocol.

The focus questions and their associated discussion and implications for a Tier 3 Validation Protocol are discussed in this section.

4.1.1 Focus Questions

1. Could a Tier 3 surrogate be used to indicate a subset of pathogens (i.e., protozoa only) and be validated to claim credit for the subset of pathogens?
    a. Responses:
        i. In general, the answer to this question was yes. Different monitoring techniques may not correlate satisfactorily with viruses but may be sensitive to protozoa removal. In this instance, it was thought appropriate that a protozoa or virus only monitoring technique could be appropriate for assurance of LRV of that pathogen group.
        ii. For some potable reuse applications, enhanced credit may only be necessary for protozoa and not for viruses, due to the ability to obtain virus credits through other mechanisms. In these situations, the default Tier 1 LRV for virus may be satisfactory.
        iii. Members of the TAC emphasized that for any pathogen analysis work, recovery should be assessed and used to adjust assay results prior to correlation.

2. Should a Tier 3 surrogate be required to be measured online such that performance can be verified in real time?
    a. Responses:
        i. With the exception of turbidity, most techniques reviewed in this report (for example PDT) are not online.
        ii. Current drafts of California direct potable reuse frameworks have focused on consistent achievement of a daily risk, as opposed to an annual risk which has previously been applied. To that end, if monitoring could be performed at a frequency to adequately verify a daily risk, then it may be appropriate to use a semi frequent monitoring technique, such as a daily pressure decay test or the sampling of an indicator to verify LRV.
        iii. A precedent was proposed as the USEPA MFGM (USEPA, 2005) which is typically performed daily, with additional PDT trigged by exceedances of turbidity in an effort to rule out turbidity false positives. Given that turbidity monitoring could be used in a similar fashion for MBRs, it may be appropriate to consider Tier 3 surrogates that are able to be monitored daily or at an increased frequency as a result of turbidity exceedances.
        iv. With the exception of severe and gross integrity failure, which would be detected by turbidity, TAC members raised the point that performance decline in MBR LRV is anticipated to be gradual with time, which supports the use of less frequent (daily), long term monitoring.
        v. TAC members also raised the point that certain techniques may be more appropriate if employed at particular points in a filtration cycle. For example, if sampling a microbial surrogate to determine membrane integrity, it may be most representative/conservative to target sampling events when the membranes are clean and at the beginning of their filtration cycle, before potential defects could be plugged with suspended solids. If a PDT is part of operation, sampling after the completion of the PDT would be another conservative approach.
        vi. The TAC was clear that at least one parameter should be continuously monitored as a means to ensure appropriate system functionality, but that the continuously monitored technique may not be the primary Tier 3 surrogate. For MBRs, the most suitable and conventional candidate is filtrate turbidity, which could be used in line with the USEPA MFGM to rapidly indicate potential issues that warrant investigation of the impact to LRV by Tier 3 surrogate analysis.
3. What are the benchmark criteria that must be demonstrated for a surrogate to be accepted for use in Tier 3 MBR validation? How does this criteria match with WaterVal or other validation approaches?
    a. Responses:
        i. The following criteria were defined:
            1. The surrogate should:
                a. Correlate with pathogen LRV.
                b. Reliably verify a minimum LRV and be responsive to treatment failures.

2. If indirect surrogates are chosen - for example indicator microorganisms or suspended solids then ideally:
    a. The surrogate would be quantifiable (detected) in the filtrate and must be quantifiable in the feed so as to obtain some measure of LRV.

ii. These criteria do match with WaterVal, however, WaterVal focused exclusively on online monitoring options. As noted previously, there are limited sensitive online monitored options for determination of LRV in MBR.

4. How many samples should be taken and over what period (i.e., should sampling cover multiple seasons or can the system be challenged and calibrated in a single day)?
    a. Response:
        i. The nature of the Tier 3 surrogate will likely determine the number of samples and time period of performance demonstration.
        ii. For example, if indicator microorganisms are selected, then:
            1. Seasonality or even diurnal variations may necessitate longer evaluation periods to capture normal variability:
                a. However, there may be limited impact of seasonality, especially on enclosed systems, isolated from the environment with stable flow, on removal barrier effectiveness. In this instance, if removal is determined (i.e., feed and filtrate sampling) then a prolonged sampling campaign may not be necessary.
            2. Composite samples could help to smooth diurnal indicator variability but were generally not recommended by the TAC as they may place indicator microorganism results out of acceptable laboratory hold times.
            3. Grab sampling was considered to be acceptable as if the performance issue was persistent, then it should be captured by infrequent grab samples.
        iii. If a barrier monitoring technique such as pressure decay testing or suspended solids removal was selected, then it should not be as sensitive to seasonal variation and could be verified with a shorter duration campaign.
        iv. In lieu of specific information, TAC members suggested that the minimum sampling frequency of 24 samples over 3 months for the Salveson et al., 2021 Tier 2 MBR validation framework may provide an adequate starting basis. After continued demonstration of reproducible validation performance, the number of samples and verification requirements could be decreased.
    b. Should the Tier 3 surrogate be validated on a site-specific basis? Alternatively, could preapproval of the surrogate be demonstrated elsewhere and more simply verified to work on a site-specific basis?
        i. Response:
            1. As with seasonality, the site-specific influencing factors would depend on the chosen Tier 3 surrogate.
            2. Even if a technique could be extensively validated offsite (for example, pressure decay testing), it was generally thought that some site-specific verification should be required to confirm adequate performance. The goal of the verification would be to demonstrate that the same relationship demonstrated during extensive offsite testing is valid under site specific conditions.

3. The concern of failure and healing modes of flat sheet vs hollow fiber membranes was raised. Although initially, the thought was that a Tier 3 surrogate could be validated independent of membrane configuration or manufacturer, there are uncertainties, and it may be warranted to require membrane product specific Tier 3 validation requirement.
4. Indirect surrogates, such as indicator organisms, would likely require site specific validations due to significant potential for operational parameters to influence their removal and also the possibility of differences in indicator feedwater abundance between sites.

5. What quality assurance (QA) / quality control (QC) requirements for pathogen/indicator analysis and surrogate technique should be established?
   a. Response:
      i. TAC members recommended careful consideration and implementation of QA procedures surrounding pathogen analysis. In particular, it was recommended that:
         1. Laboratory QA/QC requirements were established and maintained in line with best practices. Presently, the DPR 2 framework QAPP (Pecson et al., 2021) could serve as a reasonable benchmark for QA/QC requirements. In addition, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MiQE) guidelines were proposed as a benchmark where PCR based analysis of pathogens or indicator microorganisms was intended (Bustin et al. 2009). The emphasis on evaluation of laboratory QA/QC procedures was recommended to apply regardless of whether or not laboratories were following standard methods.
         2. Pathogens should be adjusted for recovery prior to calculation of LRV and/or correlation.
         3. For *Cryptosporidium* analyzed via immunomagnetic separation and staining, there were concerns that if the entire pellet volume was not analyzed then results may be uncertain. Requiring full pellet analysis of turbid waters will likely increase assay costs but was nevertheless a recommendation of the TAC.
6. What frequency should the surrogate undergo calibration/revalidation/ reverification?
   a. Response:
      i. Revalidation/calibration/verification frequency will vary depending on the chosen surrogate.
      ii. In the case of instruments, such as a turbidity meter, the minimum reactive frequency for calibration would be manufacturer specified. Ideally, a proactive calibration and verification frequency would be developed based upon continued analysis of instrument drift between calibrations. It is reasonable to assume that a turbidity meter should be verified against a calibrated benchtop unit weekly.
7. What other factors should trigger surrogate revalidation/reverification?
   a. Response:
      i. Proactive assessment of instrument and surrogate verification data would help to identify drifts from a baseline/normal operational conditions.
      ii. Quarterly sampling was thought to be a reasonable minimum for ongoing verification of an indirect surrogate such as an indicator microorganism.

iii. Significant changes in source water, a significant maintenance event, such as draining large quantities of sludge or membrane replacement, or substantial operational set point changes should trigger spot checks of the LRV/surrogate relationship to confirm that the correlation remains valid.

4.1.2 Critical Outcomes of TAC Workshop

At this time, there is insufficient data to evaluate and recommend an ideal monitoring technique that could with high resolution and sensitivity verify pathogen LRV in MBR. However, the TAC workshop did result in consensus on both the challenges and opportunities to develop a Tier 3 Validation Protocol that can provide confidence in pathogen LRV as a function of monitored parameters.

4.2 New Research by Project Partners

4.2.1 California Utility

A wastewater treatment plant, located in California, has been running a Kubota (flat sheet) MBR for more than 10 years without a single membrane replacement. This facility presented a perfect opportunity to understand the magnitude of a Tier 2 (or Tier 3) LRV of an aged membrane operated to California's "Title 22" standards (e.g., 0.2 NTU no more than 5 percent of the time within a 24-hour period and not to exceed 0.5 NTU at any time). In general, a Tier 3 validation should allow operation at control limits within the validated range such that LRV is maintained. In the example of turbidity, this may mean that turbidities higher than Tittle 22 limits could be adopted, provided it could be demonstrated that LRV was still satisfactory.

The membranes installed at the facility are the Kubota Type-515 membranes which have an average pore size of 0.2 μm. The work completed at the subject site examined *Giardia*, *Cryptosporidium*, enterovirus, adenovirus, norovirus, *C. perfringens*, somatic and male-specific coliphage, total coliforms and PMMoV. For this work, several different LRV calculation methods were investigated to determine the Tier 2 LRVs, including conventional same day Paired Sampling, random sampling from a Monte Carlo analysis (recommended in Tier 2 - Salveson et al., 2021), and Rank Order Pairing. In addition, the impact of extrapolating results below the reporting limit, by fitting detected data to an assumed lognormal probability density function, were assessed and compared with simple substitution of the reporting limit. That work has demonstrated that with less variable influent concentrations and detectable microorganism in the MBR filtrate, Monte Carlo and Paired Sampling become very similar and are preferred. The LRV results are shown in Figures 4-1 and 4-2.

The results demonstrated that where variable microorganism concentrations are present in a validation data set and there are a large proportion of non-detects, the Monte Carlo approach recommended in Salveson et al., 2021 can lead to very low 5th percentile LRVs that may not correspond to a realistic performance.

The impact of substituting data below the reporting limit was variable. In most cases, extrapolating below the reporting limit resulted in a similar or slightly higher 5th percentile LRV. This indicated that substitution of the reporting limit may be a conservative approach. The

exception was noted for enterovirus, measured by PCR. For enterovirus by PCR, the large amount of data below the detection limit and high analytical variability resulted in a higher 5th percentile LRV when the reporting limit was substituted.

Based on this study, the assumption of a lognormal distribution and extrapolation below reporting limits did not appear to negatively impact results. Extrapolation can help to include a larger proportion of experimental observations.

It is recommended that validation studies collect sufficient samples to allow comparison of multiple LRV calculation methods. If the methods converge, as in this study, it helps to provide more certainty that the claimed 5th percentile is reflective of MBR performance and is not an artefact of the calculation method.

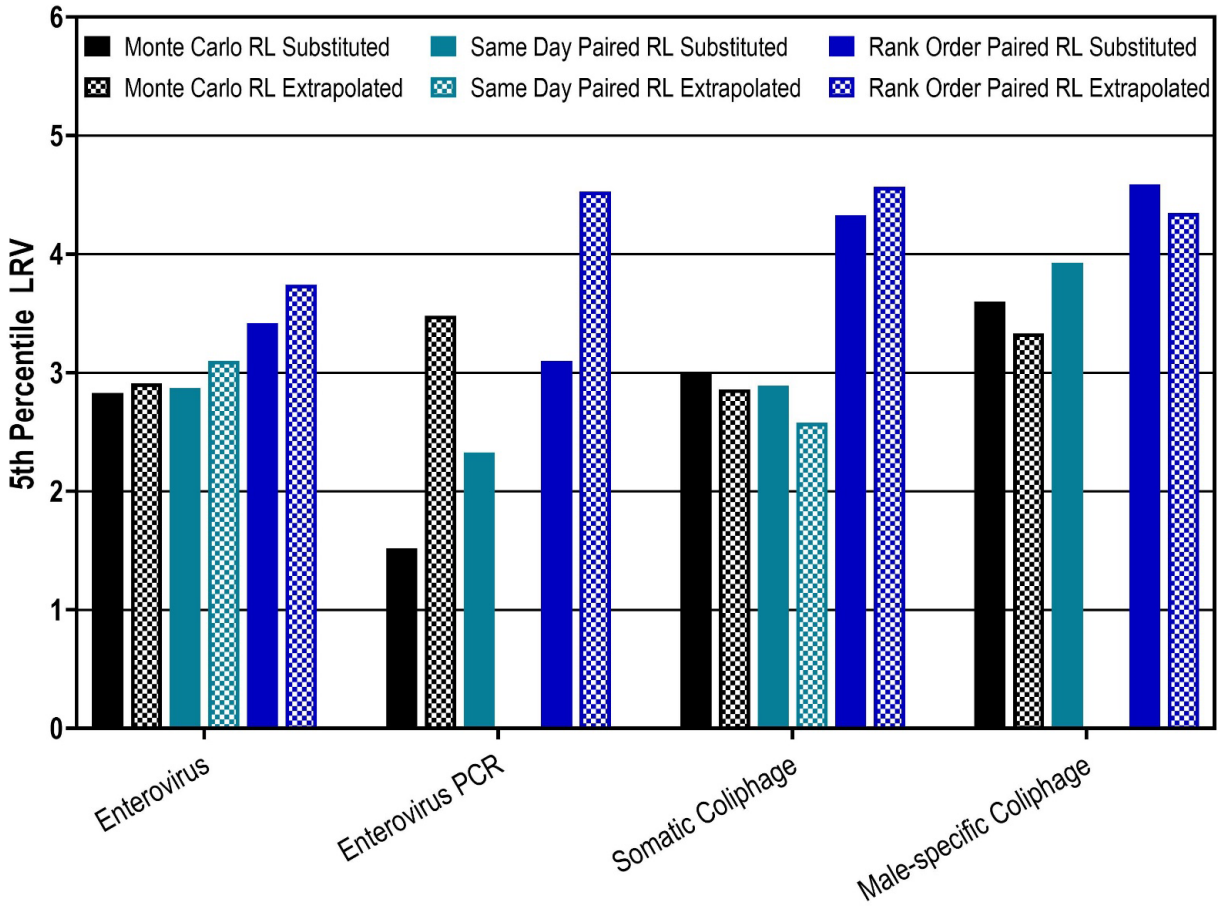


Figure 4-9. California Utility Removal of Enterovirus and Coliphage by MBR.

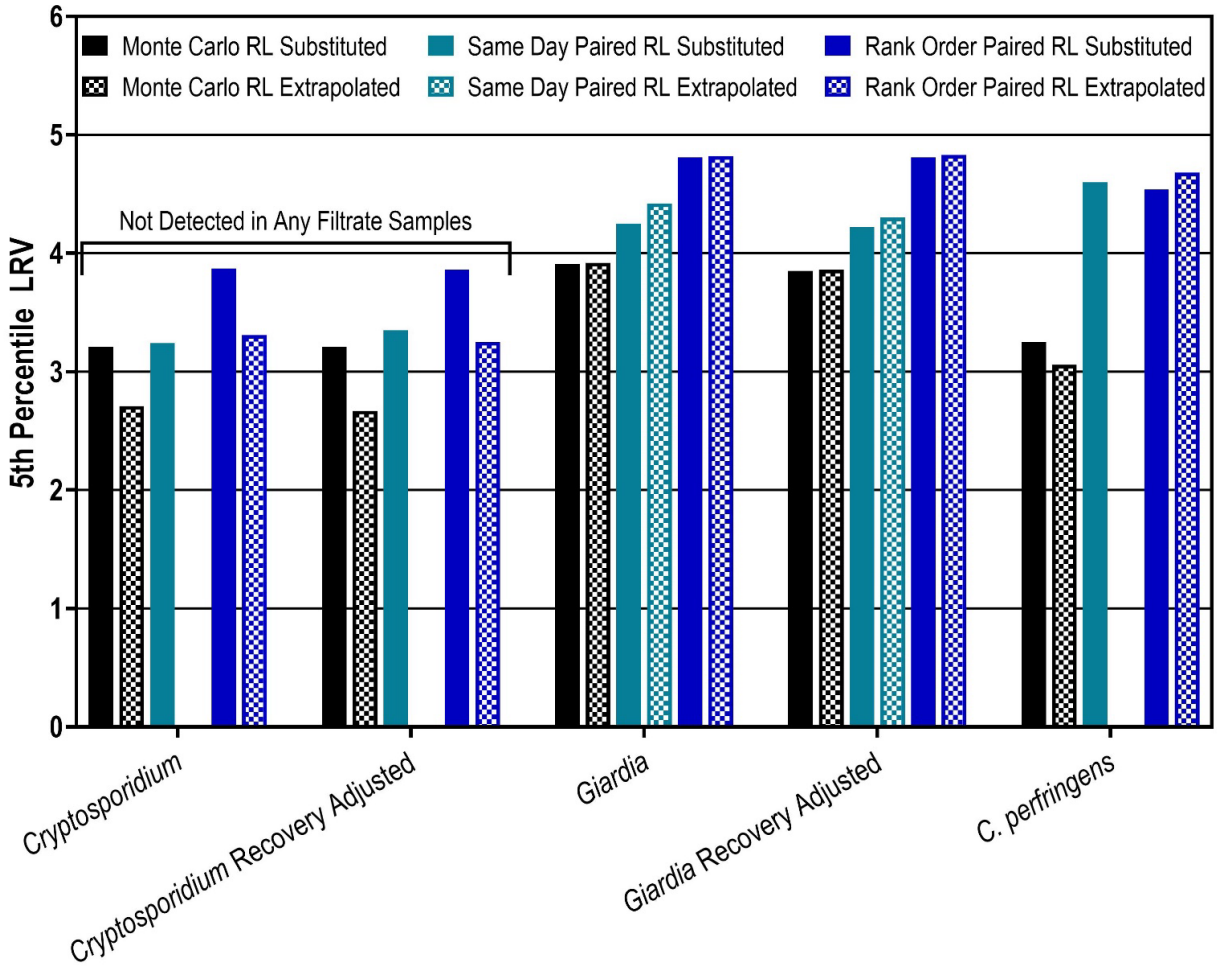


Figure 4-10. California Utility Removal of *Cryptosporidium* and *Giardia* by MBR.

This work clearly demonstrates the high LRV for both pathogens and indicators that can be obtained with both a Tier 2 and Tier 3 validation, noting that the California utility work was entirely a Tier 2 effort and the direct correlation between a monitored parameter and a specific LRV, which would be Tier 3, was not demonstrated at this site.

4.2.2 Northern NSW Australia

While the California utility work summarized above did not attempt to correlate MBR filtrate turbidity with a specific LRV, new work out of Australia demonstrates this correlation, resulting in a Tier 3 analysis. As mentioned previously, the complexity of pathogen removal mechanisms in MBR has, until now, prevented development of a rigorous CCP based method to validate treatment performance. This new work utilizes prior WaterVal data (WaterSecure, 2017) and new pathogen removal at an anonymous full-scale MBR in Northern NSW Australia with ~8-year-old flat sheet membranes. The data presented below is the first to confirm the logarithmic relationship between filtrate turbidity and LRV in MBR at a full-scale facility. According to both the model and full-scale data, relatively high turbidities (>>0.5 NTU) are needed in order to observe low indicator LRVs (< 2).

Accordingly, the aim of this new work was to assess the correlation of turbidity with MLSS, identify the potential for correlation of loss of indicator LRV with turbidity and propose a method for setting CCLs of a turbidity meter to assure appropriate LRV during full-scale MBR operation. In addition, the empirical model developed at bench scale was compared to full scale MBR results where it was possible to sample from individual filtration units with differing levels of membrane integrity, and filtrate turbidity.

4.2.2.1 Bench Work and Modeling from WaterVal

The modelling work conducted as part of WaterVal was described in Section 2.5.2 and was applied as part of this work.

The data from the site studied was revisited and an LRV_{Bio} was determined using Monte Carlo calculation techniques using Equation 2-2 and the data from WaterVal are reported in Table 4-1.

Table 4-10. LRV_{Bio} from the Full WaterVal Data Set and for the Specific Site Studied in this Chapter.

Indicator	LRV_{Bio} PDF Parameters (Entire Data Set)		
	5th Percentile	Median	95th Percentile
C. perfringens	-2.0	-1.4	-0.7
E. coli	-0.2	0.9	2.0
Somatic coliphage	-1.7	0.0	1.6
FRNA bacteriophage	-0.7	1.1	2.8
Indicator	**LRV_{Bio} PDF Parameters (Full Scale Study Site)**		
	5th Percentile	**Median**	**95th Percentile**
C. perfringens	-1.8	-1.5	-1.2
Somatic coliphage	-1.4	-0.6	0.2

The model was then developed for this site using Equation 2-9. In addition, an optimized LRV_{Bio} was calculated to minimize the root mean squared error (RMSE).

Using the above information, log removal of the indicator organisms was modeled (Using Equation 2-9) to predict LRV as a function of turbidity, which is included in the information presented below.

4.2.2.2 Full Scale Evaluation

Permeate Partners (Australia) led the new work in Northern NSW Australia. For this effort, raw sewage and MBR filtrate samples were collected at strategic locations from, the combined membrane trains, each membrane train and from individual membrane filtration units. These samples were analyzed for the indicator microorganisms *C. perfringens* and somatic coliphages. For each sample, the LRV from raw sewage was determined and filtrate turbidity data was collected. Individual membrane units were able to be sampled from the camlock fittings, which allows for much greater resolution of performance on specific damaged membranes.

The LRVs of *C. perfringens* and somatic coliphage calculated for a range of turbidities observed during full scale site sampling are shown in the Figures below. In addition, the model predicted

relationship using site specific data was overlayed. Finally, a model optimized LRV_{Bio} was used to fit the results. For the optimized model, the value of LRV_{Bio} was adjusted to minimize the root mean squared error between the actual observed LRV and turbidity data and the model.

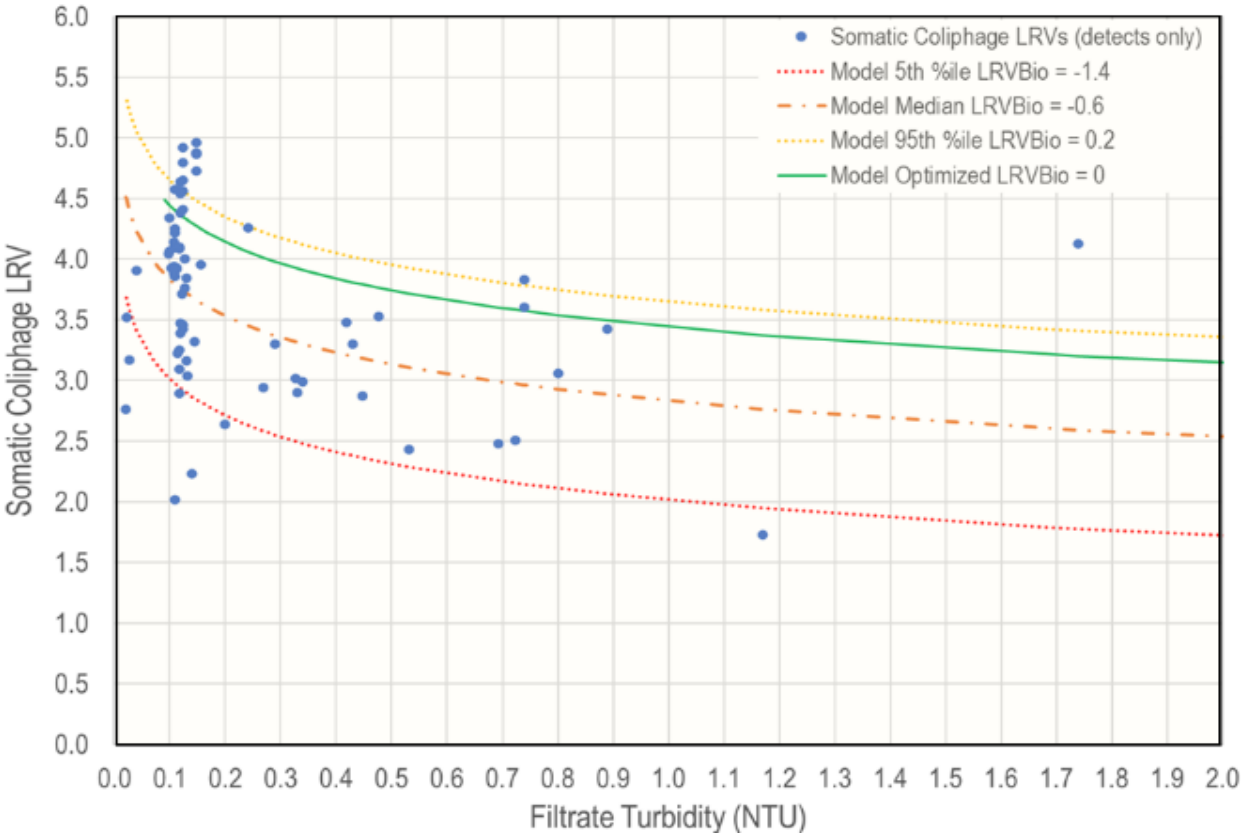


Figure 4-11. Full Scale LRV Sampling and Turbidity Measurement (blue dots) Shown Relative to the Model Prediction (lines) using LRV_{Bio} Statistics and also an Optimized LRV_{Bio} to Fit the Data (green line) for Somatic Coliphage.

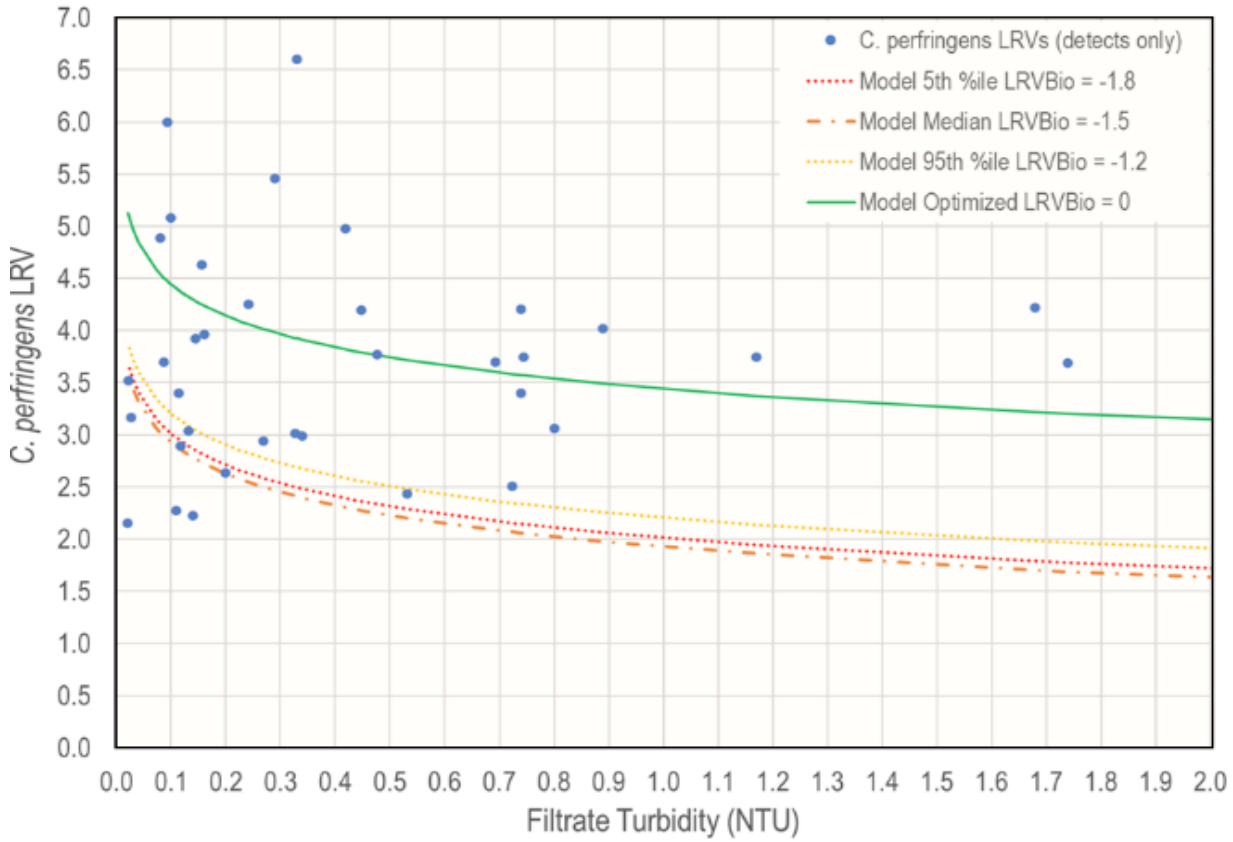


Figure 4-12. Full Scale LRV Sampling and Turbidity Measurement (blue dots) Shown Relative to the Model Prediction (lines) using LRV_{Bio} Statistics and also an Optimized LRV_{Bio} to Fit the Data (green line) for *C. perfringens.*

The results indicated that:

- Use of the 5th percentile LRV_{Bio} provided a conservative estimate of removal as the LRV/Turbidity model typically underestimated relative to actual observed LRV. For both of these indicators, the LRV_{Bio} was negative which indicates that there was potential for some accumulation within the bioreactor.
- For somatic coliphage, a large proportion of data between 0.1 NTU and 0.2 NTU showed a high variability of LRV, ranging from 3 - 5. This might suggest that the onsite turbidity meter was only capable of showing significant performance decline for this virus indicator at turbidities higher than 0.2 NTU.
- The optimized model resulted in an LRV_{Bio} of 0 for both organisms. This implied that the bioreactor term might not improve the prediction or relation of turbidity with LRV and that simply plotting LRV vs log (MLSS/turbidity) may provide a sufficient estimate. The RMSE for the optimized model was 0.9 and 1.2 for somatic coliphage and *C. perfringens*, respectively.

Full scale MBR sampling demonstrated that the logarithmic relationship between LRV and filtrate turbidity proposed at bench scale during WaterVal (Section 2.5.2) could be replicated. This data is significant, as it is the one of the first demonstrations of how a site-specific correlation of turbidity and LRV could be achieved at full scale to set meaningful CCLs.

If approach 1 is followed and the 5th percentile LRV_{Bio} is used, the results from the case study above suggest that use of turbidity with predict conservative and low LRVs. Further work is required to understand the discrepancy and limited significance of the LRV_{Bio} term proposed in Equation 2-9.

4.2.3 Metropolitan Water District of Southern California

The Metropolitan Water District of Southern California (MWDSC) has now completed more than a year of extensive MBR challenge testing and analysis at their Advanced Purification Center (APC). A detailed test plan is available that describe a significant analytical effort to evaluate Tertiary MBR (an MBR treating secondary effluent) (MWDSC/LACSD, 2019), which is near completion and secondary MBR which is to commence in 2022.

The test plan indicates that over 70 samples will be taken for *Cryptosporidium* and *Giardia* as well as indicator organisms such as total coliforms and *C. perfringens*. Samples will be taken for a range of damage conditions.

The very large amount of data collected by the test plan over a number of different membrane integrity conditions may be the first instance where pathogen data is available to evaluate the potential for correlation of surrogates such as PDT and turbidity with pathogen and indicator microorganism LRV.

A number of preliminary findings for the extensive tertiary MBR validation program that are relevant to this work were presented recently (Lehman et al., 2022). In particular, a novel approach for using Monte Carlo simulation of bins of filtrate/operating data was used to determine 5th percentile LRVs of *Cryptosporidium* and *Giardia* under a range of operational and membrane damage conditions. This approach was considered necessary as a means to account for the blend of very large volume (approximately 10,000 L) filtrate samples of pathogens, collected over 16 hours, PDT results conducted at the start of a collection cycle and filtrate turbidity monitored online over the duration of sample collection. The project highlighted the very important and practical challenges with trying to correlate surrogates and pathogens measured over different time scales (i.e., 16 hours vs. daily (24 hour) intervals) which is illustrated in Figure 4-5 below. Preliminary study results also suggest that shorter sample collection periods for pathogens and indicator organisms may further confound data analysis and interpretation compared to daily turbidity values. In addition, 24-hour sample collection and correlation periods are considered more appropriate for assessment of daily risk which is a target of recent drafts of DPR guidelines for California.

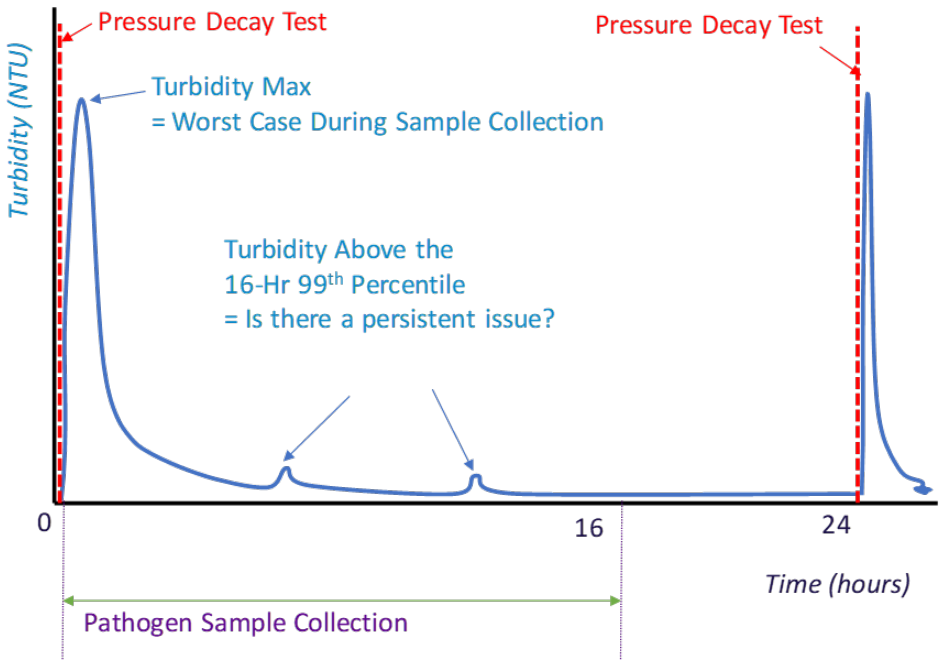


Figure 4-13. Illustration of the General Trend in Results and the use of Statistics to Relate Both Immediate Online Monitoring Response and Long-Term Behavior (via the 99th Percentile) to Pathogen Sampling.
Note that the project reported an increase in turbidity following PDTs when fibers were intentionally cut and there is some concern that frequent use of PDT may be deleterious to water quality (adapted with permission from Lehman et al., 2022).

The results for each bin are summarized in Table 4-2 below.

Table 4-11. LRV and Surrogate Results Analyzed by a Novel Binning Approach Reported for a Tertiary MBR (MBR Treating Secondary Effluent).
Results adapted with permission from Lehman et al., 2022.

Pathogen LRVs	Bin 1 5th Percentile LRV (N=25)	Bin 2 5th Percentile LRV (N=25)	Bin 3 5th Percentile LRV (N=26)
Giardia	4.7	3.5	3.3
Cryptosporidium	4.1	3.3	3.0
Primary Surrogate - Turbidity	**Bin 1 Value**	**Bin 2 Value**	**Bin 3 Value**
Max Sample Day Max Turbidity (NTU)	0.1	0.2	1.6
Max Sample Day 99th Percentile Turbidity (NTU)	0.06	0.09	0.20
Secondary Surrogates	**Bin 1 Value**	**Bin 2 Value**	**Bin 3 Value**
95th Percentile Filtrate *C. perfringens* (CFU/L)	0.15	0.57	3.1
95th Percentile Filtrate *E. coli* (MPN/L)	13	21	43
95th Percentile Filtrate Total Coliform (MPN/L)	85	120	300
Max PDT Result (psi/min)	0.77	1.5	2.0
Abbreviations: CFU/L - colony forming units per liter; MPN/L - most probable number per liter; psi/min - pounds per square inch per minute.			

From the extensive validation testing results gathered, limits were proposed for each of the bins above. The operational monitoring intent would be to claim the validated LRVs based on

remaining within the limits outlined for turbidity. PDTs could then be used to rapidly diagnose turbidity events to determine if there was membrane integrity failure; a train could be diverted while indicator organisms or pathogens were tested in order to quantify a meaningful LRV. The pragmatic approach uses the best available monitoring approaches at this time, outlines a means to respond to and isolate potential gross integrity failures in less than 15 minutes, and is supported by a significant collection of data. In lieu of an established Tier 3 protocol, the MWDSC project appears to outline the current best practice for correlating pathogen LRV with monitoring techniques. In addition, the MWDSC study demonstrates that significant membrane damage appears to be necessary to reduce protozoa LRVs calculated on a daily basis to less than 3-log. For a system to produce water with less than 3-log protozoa removal, filtrate turbidity and PDT results are easily identifiable as outliers and higher than would be considered acceptable.

4.3 Tier 3 Validation Protocol Outline

The information from the workshop above, coupled with the other work presented herein, was used to develop a Tier 3 Validation Protocol Outline.

With respect to prior WaterVal framework, this proposed Tier 3 protocol does deviate by removing the requirement that Tier 3 surrogates are measured online. Instead, this protocol suggests that a Tier 3 surrogate should meet the MFGM DIT requirement of at least daily measurement and corrective action. Daily measurement and corrective action is considered acceptable in order to maintain a daily health risk. If a daily surrogate is selected for correlation, then an online indirect integrity monitoring technique should also be selected to provide rapid response to any gross failures. Turbidity is considered an appropriate technique for this purpose with either adoption of validated limits or local guidance values, such as the 0.2 NTU 95% of the time criteria. Based upon the limited available validation data, the project team cannot be confident that a Tier 3 validation is agnostic of supplier or operational parameters. Accordingly, at this time, a Tier 3 validation is assumed to be supplier and site specific.

4.3.1 Section 1 - Facility Description and End Use

- Required monitoring frequency with respect to end use health risk:
  - IPR - Goal is to meet an annual risk target.
  - DPR - Goal is to meet a daily risk target.
- Define the value of Tier 3:
  - To reduce treatment barriers through greater confidence in MBR LRV?
  - To provide LRVs in excess of regulated levels and higher than that possible with Tier 1 or Tier 2?
  - Define how a Tier 3 LRV provides greater benefit to a sponsor utility compared to a Tier 2 LRV.

4.3.2 Section 2 - Proposed Tier 3 Surrogates and Indicators

- The ideal surrogate's characteristics include:
  - Measurable in the required process streams.
  - Has a proven and repeatable analytical technique, with well-defined quality control criteria.
  - Conservatively measures performance (e.g., exceeds a set surrogate threshold prior to exceeding a set pathogen threshold).
  - Has been demonstrated to vary in a statistically significant manner with LRV for the membrane product and operational range.
- The ideal indicator's characteristics include:
  - Measurable in the influent and effluent. Ideally the measurement sensitivity and/or available concentrations will allow demonstration of high LRVs that are representative of system performance.
  - Has a proven and repeatable analytical technique, with well-defined quality control criteria.
  - Conservatively measures performance (e.g., shows equal or lower LRV than the target pathogens for the range of interest).
  - Can be justified to be physically representative or conservative with respect to pathogen removal (i.e., has a similar or smaller size or may exhibit a higher tolerance to other removal mechanisms such as biodegradation).
  - Has been demonstrated to vary in a statistically significant manner with LRV for the membrane product and operational range.

4.3.3 Section 3 - Test Plan for Tier 3 Surrogate Validation

- Define the operational window of the MBR:
  - Feed water quality (e.g., BOD, ammonia, TSS).
  - Operational variables (e.g., SRT, HRT).
  - Effluent quality goals (e.g., turbidity, nutrient removal).
  - Damage of the membrane (e.g., no damage (baseline) versus impaired conditions):
  - Maintenance Cleans (MC) and Recovery Cleans (RC), type and interval.
  - Note that at this stage there is uncertainty as to if change of operational parameters within the ranges outlined in Tables 3-4 and 3-5 will impact LRV of pathogens. Some work was conducted as part of WaterVal and published in (Branch et al., 2021) but was limited to indicator organisms. A clear research need is systematic and representative studies to distinguish which operational parameters significantly influence LRV of pathogens.
- Determine what surrogates and indicators to use (per above), with examples including:
  - Surrogates: Turbidity, PDT, TSS.
  - Indicators: *C. perfringens*, Total coliforms, fecal coliforms, *E. coli*, coliphage (somatic and male specific), PMMoV.

- Determine what pathogens to evaluate. Recommended target pathogens from Salveson et al., 2021 include:
  - Protozoa: *Cryptosporidium*, and *Giardia* if specific LRVs are sought for each protozoa.
  - Virus: enterovirus.
  - Further research may identify that other pathogens are more resistant to treatment, in which case these should be used as target pathogens.
- Define test conditions:
  - Baseline. No intentional impairment of membranes. Could utilize old or new membranes for testing, noting that testing new membranes alone does not provide conservative future results for long term damaged conditions.
  - Impaired. Impairment of membranes (e.g., fiber cutting) appears necessary to generate a range of effluent quality (e.g., higher turbidity, higher TSS, higher PDT), which then elicits a measurable LRV response or sets a threshold for performance.
  - Testing of old and new membranes within the same operational conditions, without intentional damage, may be acceptable if a measurable difference in both surrogate, indicator and pathogen LRVs can be achieved.
- Define sample timing and duration:
  - The sample timing and duration may have impacts on the final LRV credit and ultimate pathogen monitoring approach. Two approaches are considered below:
    - Approach 1: Sample immediately after a disruptive event such as a MC or RC or PDT to determine the worst-case performance; or
    - Approach 2: Sample after the passage of a disruptive event, understanding that diversion of flow during disruptive events would be required going forward after such testing.
    - Approach 1: Would likely result in very conservative LRV credits that would understate treatment performance a majority of the time. Approach 2 may demonstrate higher LRV credits but could then require a higher number of diversions to remain within the monitoring parameters and as a consequence result in a degree of wasted water. Crediting conservatively based on Approach 1 may permit more flexibility to divert less, with the consequence of lower LRVs.
  - Collect large samples for increased analytical sensitivity, and sample for the same duration of time for all testing (e.g., 1,000 - 10,000-liter samples) Collection of larger samples may necessitate either bulk collection in large storage tanks or composite collection through filters (e.g., e=Envirochek HV filters for *Cryptosporidium* and *Giardia*) over a long period 8 - 24 hours.
  - Collect samples for all parameters over the same time interval (e.g., do NOT collect and compare a grab sample indicator result with a composite pathogen sample collected over 8 - 24 hours). This may involve averaging, selecting adequate statistics or attempting to maintain very stable operational parameters over the sample collection period in an effort to compare results to these.

- Define sample replication:
  - For each test condition (e.g., Baseline or Impaired), conduct a minimum of 20 samples to allow calculation of a 5th percentile LRV. It is recommended that a longer-term sampling campaign is conducted to account for temporal variability. A minimum total sampling period of 3 months is recommended as part of Tier 2 testing in Salveson et al., 2021.
  - If the variation in surrogate parameters is not sufficient, further impairment or longer-term monitoring may be necessary to develop a meaningful correlation.
- Determine membrane repair characteristics:
  - Document MBR filtrate turbidity immediately after a disruptive event, and the time necessary to reach "baseline" turbidity.
  - Evaluate pathogen, indicator, and surrogate data during disruptive events (e.g., minutes) and compare performance over longer-term sampling (e.g., hours, but no more than 24 hours).
- Define QA/QC procedures (e.g., spiked recovery) for all pathogens and surrogates BEFORE detailed testing occurs.

4.3.4 Section 4 - Data Analysis

- Use of QA/QC for all pathogens and indicators:
  - Unless demonstrated to be acceptable otherwise, perform spiked recovery tests for each pathogen and indicator for each sample.
  - Demonstrate a reasonably consistent recovery, refining methods if needed.
- Perform a statistical analysis of data sets using the following potential methods.
  - Microorganisms - evaluate the lower 5th percentile for each test condition using both same day paired LRV and monte Carlo based assessment of LRV. Per Salveson et al., 2021, the monte carlo calculated 5th percentile will likely form the basis of the LRV credit, however, comparison with same day paired results may help to check if the calculated LRV is subject to artifacts as a result of analytical variability.
  - Online surrogates (e.g., turbidity):
    - If collecting pathogen samples over a long period it may be necessary to correlate sampling results with a statistical measure of all filtrate turbidity results over the same period. For example, the maximum, 99th and 95th percentile turbidity may be more appropriate measures to summarize variability across the sampling time.
    - For PDT, the average PDT result before and after sampling could be used to indicate the relative level of membrane damage during prolonged sample collection.

4.3.5 Section 5 - Setting Tier 3 Operating LRV

- Determination of Surrogate operating LRV and associated pathogen LRV.
- Quantification of Surrogate operating limits (Hypothetical example - > 0.5 NTU, Turbidity high alarm corresponds to LRV virus = 2.0, Turbidity = 1.0 NTU high alarm LRV Virus < 1.5 and corrective action is required).
- Recommendation for re-verification/ongoing verification of surrogate LRV relationship adequacy.
- Recommendation for ongoing surrogate calibration.

4.4 Research Needs to Support MBR Validation

The Tier 3 validation framework outlined in this report makes conservative assumptions regarding the number of samples and limitations of applied validation results (i.e., supplier and site-specific validation) in an effort to compensate for a lack of clear evidence to justify a specific validation approach. This section summarizes the research teams perspectives on potential avenues to arrive at a more appropriate and ultimately adoptable MBR validation framework.

4.4.1 Monitoring Techniques

The ideal monitoring technique/parameter will be a single or combined variable which can be related via equations to the LRV of target pathogens. This may take the form of a single surrogate which could be measured and acted upon at a sufficient frequency, i.e., at least daily and correlates with pathogen LRV. Research to evaluate new monitoring techniques for MBR validation should strive to achieve the following:

- Demonstrate a correlation with pathogen LRV.
- Demonstrate under which operational conditions or water qualities the correlation holds.
- Identify if there are MBR configuration or product specific limitations for application of the monitoring technique.
- Describe the removal mechanisms the technique is able to indicate.

4.4.2 Pathogen Sampling and Correlation

It is challenging to quantify LRVs around MBR. Pathogens are typically at low concentrations in wastewater and in order to quantify them in filtrate large sample volumes need to be taken. Table 4-3 provides estimates of the sample volumes required to demonstrate 4-log of protozoa and 3-log of virus removal by MBR. The long period of sample collection (possibly 12 hours for *Cryptosporidium* and *Giardia*) can create uncertainty on how to manage, report and correlate operational and monitoring parameters with the water quality.

Table 4-12. Anticipated Range of MBR Microorganisms and Required Sample Volumes to Observe 10 Organisms.
Note actual sample volumes will be determined based on method development during commissioning.

Microorganism	Matrix[1]	Log10 (Organisms/L)		Minimum Sample Volume (L)[2]	Raw WW Data Reference
		5th Percentile	95th Percentile		
Cryptosporidium	Raw WW	1.0	2.4	0.9	DPR2 - (Pecson et al., 2021)
	MBR Filtrate	-3.0	-1.6	9,200	
Giardia	Raw WW	3.3	4.7	5.0×10^{-3}	
	MBR Filtrate	-0.7	0.7	46	
Enterovirus	Raw WW	1.5	4.9	0.3	
	MBR Filtrate	-2.5	1.9	290	
C. perfringens	Raw WW	3.8	7.0	2.0×10^{-3}	(Fontaine and Morris, 2020)
	MBR Filtrate	-0.2	3.0	17	
Somatic Coliphages	Raw WW	5.1	7.3	8.0×10^{-5}	
	MBR Filtrate	2.1	4.3	0.08	
Male-specific Coliphages	Raw WW	4.5	7.0	3.0×10^{-4}	(Salveson et al., 2021)
	MBR Filtrate	1.5	3.0	0.3	

Notes:
1. Virus MBR filtrate densities calculated assuming 3 LRV from feedwater results, Protozoa assumes 4 LRV from feedwater.
2. Calculated targeting observation of 10 organisms at the 5th percentile concentration.

Abbreviations: WW - wastewater.

Guidance is specifically needed for the following areas:

- Best practice strategies for correlating instantaneous monitoring parameters with a composite sample collection.
- A justification and sound approach which defines the number of samples required to adequately form a correlation. The 20 samples suggested in this work is related to Tier 2 guidance and is the number of samples necessary to calculate a 5th percentile.

4.4.3 Systematic Studies at Scale to Relate Pathogen Removal Trends and Operational Conditions

The work performed to date to evaluate relationships between MBR LRV and operational conditions has been limited to indicator microorganisms only. A larger data set containing pathogen data would be required to verify the impact of operating conditions on LRV. Distinguishing which operational conditions significantly influence pathogen LRV is an important step as it will help to set boundary conditions under which both Tier 3 and Tier 2 testing can be conducted, thereby reducing the site-specific sample burdens. The emphasis on Scale and not benchtop data is made here as benchtop MBR systems are rarely able to be operated under conditions that appropriately represent the complex design operation and mixing experienced at scale. At a minimum, studies would feature at least one MBR production element.

4.4.4 Continual Improvement and Review of MBR Pathogen Data

Ideally a public domain database would be collated and maintained to allow a broader assessment of relationships between MBR LRV and operation and monitoring data. This data base could be used to revise Tier 1 LRV targets and with a larger pool of operational parameters it may be possible to define global correlations and justify Tier 3 monitoring strategies. To be successful, minimum quality criteria would need to be established for such a data base including:

- Documentation of pathogen QA/QC (recovery, method and other data quality indicators).
- Documentation and results for monitoring systems (e.g., turbidity meters) as well as the membrane product.
- Reporting of operating parameters shown to influence pathogen LRV (See Section 4.4.3).

4.5 Tier 3 Validation Protocol Conclusions

Turbidity is the only available online monitoring option to verify the membrane barrier is intact in an MBR. The available information to date suggests that very high turbidities are required for a significant decrease in LRV and that there is uncertainty about LRV at very low turbidities. Turbidity may be useful for rapidly indicating gross integrity failure but may need to be supported through the use of offline surrogates or indicator microorganisms to rule out false positives.

With very few sources of pathogen data correlated with surrogates and indicator microorganisms it is not possible to specify with certainty whether:

- Tier 3 validation should be membrane product independent and,
- If fewer samples than that recommended in Salveson et al., 2021 Tier 2 validation should be taken. To that end, the protocol above suggests at least 20 samples per correlation point, which means that the initial sampling burden of Tier 3 is twice that required for Tier 2.

With currently available data and monitoring practices a Tier 3 validation will likely be more costly than Tier 2 and not necessarily result in a higher LRV credit. The principal benefits of Tier 3 validation would be:

- To allow faster response time to failures,
- Provide more certainty on the LRV achieved at different surrogate trigger levels; and
- To provide operational flexibility and potentially justify operation at higher surrogate limits that are potentially associated with lower LRVs - thereby reducing potential for diversions and produced water losses.

Based on utility and supplier feedback, Tier 3 validation is unlikely to be pursued until case studies are available that demonstrate the cost and benefits. Tier 2 validation as described in Salveson et al., 2021 is expected to provide sufficient additional LRV sufficient for DPR applications.

There is potential that with further research to address some of the uncertainties raised above, economical validation of MBR systems may be able to be achieved.

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