Australian Water Recycling
Centre of Excellence

Project Report

National Validation Guidelines for Water Recycling: Membrane Bioreactors

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National Validation Guidelines for Water Recycling: Membrane Bioreactors

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Executive Summary

Scope	1. NatVal Subproject 1 (SP1) was created in order to develop appropriate validation guidelines for Membrane Bioreactors (MBRs) in Australia. 2. SP1 conducted a critical review of current literature on LRV (n > 1000 LRV data points obtained) in MBR, validation reports/guidelines, and a sampling campaign with a total of 180 visits (5 indicators investigated paired with operational data) to 11 different full scale MBRs in order to create a database of MBR performance and operation. Bayesian Belief Networks, created and trained on the data collected, were used to identify significant influencing factors. The new data obtained, combined with the assessment of current validation practice, was used to populate the 9-point-validation protocol template provided by the protocol development group.
Use of this report	3. Outputs of this research have been used in the development of proposed validation guidelines for MBRs. This document serves as a summary, cross referenced to appendices; to highlight the relationship between SP1 research outputs and the final proposed validation guidelines.
Stakeholder Concerns	4. The uncertainty as to whether an MBR can be accredited to the required LRV of a scheme presents significant financial uncertainty for suppliers and designers. In at least one instance, no efforts were made to validate a MBR due to fears that overall scheme delivery would be slowed. Instead, an ultrafiltration unit was placed after the MBR, essentially introducing 100% membrane redundancy and resulting in an increase CAPEX and in energy consumption estimated at 30%. 5. Only one state based validation guideline developed by the Victorian Department of Health exists for MBR in Australia. The industry perspective is that this guideline is conservative and as a result is not feasible to implement, especially for smaller schemes. Regardless, the fact that only one state based guideline exists is evidence that there is insufficient guidance in other states, which has led to highly inconsistent, case by case, accreditation of MBRs in Australia. As a result, health risk has not been appreciated equally. 6. From the regulators perspective, there is significant uncertainty on the effect and significance of operational parameters on, and the capacity of online monitoring options to correlate with, LRV from MBR. In addition, there is still limited available data on the suitability of surrogates used for performance monitoring with respect to target pathogens.
Aims & Objectives	7. The overall aim of this project was to develop validation protocols for MBRs in water recycling schemes. In order to achieve this multiple objectives were determined: 8. Objective 1. Collect data from literature, existing validation reports/guidelines and sampling activities in order to identify the LRV applicable to MBR and the mechanisms responsible, identify significant factors that influence LRV and to establish the current practice for MBR validation in Australia. 9. Objective 2. Perform multivariate analysis, including the use of Bayesian Belief Networks, to decompose the complex relationships between operational parameters in order to determine factors that significantly influence LRV. 10. Objective 3. Assess the potential for online monitoring to correlate with LRV in MBR in order to provide continual assurance. 11. Objective 4. Document and quantify the impact of various hazardous events that could lead to diminished LRV in MBRs including integrity failure and shock loading as well as events that occur during operation such as ageing of membranes and chemical cleaning. 12. Objective 5. Translate the evidence based conclusions from research outputs, as well as the perspectives gained from review of current practice' into appropriate validation guidelines for MBR, consistent with the 9-point framework outlined by the Protocol Development Group specified in Appendix A Section 1.

Review of MBR literature on LRV and Online Monitoring (Sections 1 – 3)	13. Published scientific literature was evaluated in order to identify the mechanisms and expected performance of pathogen removal in MBRs as well as potential online monitoring strategies. 14. The removal mechanism in MBRs are pathogen specific and include: 1) size exclusion by the clean membrane, 2) adsorption to suspended solids (MLSS) increasing the effective particle size and removal in waste activated sludge, 3) exclusion by the fouling layer and 4) biological predation. 15. For pathogens larger than the membrane pore size, typically 0.04 – 0.4 µm in MBR, size exclusion is the predominant mechanism. For viruses, typically < 0.1 µm, in the order of the membrane pore size rejection is enhanced, greater than that expected with a clean membrane, due to the dynamic fouling layer but also a higher tendency to adsorb to MLSS. For this reason, there is limited evidence of significant differences in virus removal due to pore size in full scale MBRs. 16. It is not typical for all pathogens to accumulate within the bioreactor in an MBR after being rejected by the membrane for two reasons: (1) biological predation will occur to some extent and (2) overall accumulation can be limited through sludge wasting (ie proportional to solids retention time). 17. Turbidity is the most convenient online monitoring technique to infer membrane integrity and hence pathogen removal in MBR. Turbidity measures light (or laser) scatter at 90°, proportional to the amount of suspended solids in a solution. An MBR contains a significant amount of MLSS adjacent the membrane (2,000 – 14,000 mg/L). As a result, significant loss of membrane integrity should result in spikes in turbidity due to transfer of detectable quantities of SS. At this point, corrective actions such as diversion of product water could take place automatically to protect against loss of containment of pathogens. 18. Direct membrane integrity testing techniques, such as pressure decay testing (PDT), are not favoured in MBR due to the difficulty in maintaining control PDT due to the harsh operating environment, the limitation to specific membrane configurations (certain hollow fibre and tubular, not flat sheet) and the lack of correlation between PDT and LRV in MBR; due to the action of mechanisms other than pure size exclusion. 19. Even though more than 1000 LRV data points had been reported in over 30 published papers for MBR in the last 20 years, limited corresponding operational data was reported. As a result, no correlations or identification of statistically significant operating parameters could be made directly from literature alone.
Review of Current Validation Practices (Sections 5)	20. Key elements were evaluated from the Victorian validation guidelines (VDoH 2013) and also from two validation reports, two recycled water quality management plans and one set of validation testing results. 21. Turbidity was the chosen monitoring technology in all cases. For one report, an attempt was made to correlate turbidity with MLSS and hence achievable LRV, as recommended in VDoH (2013). 22. Operating parameters were documented in most reports, however justification of their influence on LRV was limited or non-existent. 23. Default or indicative values for LRV in MBR were claimed based on direct microfiltration listed in the Australian Guidelines for Water Recycling Phase 1 2006, Table 3.8, for two of five sites. No indicative value is listed for MBR in VDoH (2013), although there is one for activated sludge alone. 24. Three of the five sites conducted challenge testing, the indicators tested for virus, bacteria and protozoa were predominantly somatic coliphages (FRNA bacteriophage at 1 site), *Escherichia coli* and *Clostridium perfringens*, respectively. These indicators were consistent with surrogates listed in VDoH (2013). However, no attempt was made to correlate the use of these indicators with target pathogens, enteroviruses and cryptosporidium as listed in VDoH (2013). Sampling frequency and period was less than that recommended in VDoH (2013), and also different in all cases, with total number of sampling events varying between 14 and 30 over a period of 7 to 14 weeks. The VDoH

	(2013) recommends analysis a 3 different fouling conditions, at 3 points in the filtration cycle for 6 consecutive cycles each on non-consecutive days, spread over extreme seasonal periods unless a worst case period can be justified. This equates to a minimum of 54 samples taken over a year. One site did not need to conduct challenge testing given that it provided literature for performance of the membranes and had historic challenge test data on the activated sludge plant that was upgraded.
Sampling and Analysis of Full Scale Site Data (Sections 6 and 7)	25. No adequate data set, containing both microorganism removal and operational parameters, was available to allow correlation and determination of influencing factors on LRV. MBR removal mechanisms are complex and synergistic, leading to difficulties when applying simplistic modelling approaches. 26. A corresponding set of LRV for virus (somatic coliphage, FRNA bacteriophage), bacteria (*E. coli*) and protozoan (*C. perfringens*) was collected alongside shortlisted operational and monitoring parameters during a sampling campaign across 11 full scale MBRs for a total of 180 site visits. Bayesian belief networks were constructed to elucidate significant relationships and determine the influence of parameters. 27. From a preliminary analysis operation under the following conditions was confirmed to lead to a higher likelihood of a poor LRV: low HRT, high flux, high permeability, low TMP, high permeate turbidity, low MLSS and high dissolved oxygen. These conditions were identified and used to define an operational envelope for validation testing. 28. The results from Bayesian analysis are presently undergoing further review and model refinement, with a final model to be proposed by end 2015.
Consequences of Hazardous events on MBR LRV (Sections 4, 8, 9, 11, 12 and A1)	29. Consideration of hazardous events and likely monitoring/control strategies was deemed important, in order to support on-going validation of MBR systems. Chemical cleaning and membrane ageing were included in the consideration of hazardous events due to the perceived consequence of both in affecting removal by the membrane. 30. Potential consequences of hazardous events were scoped in Section 4. Chemical cleaning was assessed at a full-scale site in Section 8. The condition of MBR membranes at 10 years was compared before and after total replacement in Section 9. The impacts of hazardous events on bulk parameters and LRV were reported from pilot scale assessment in Sections 11 and 12. An overview matrix of other process failures from pilot testing and full scale site investigation was provided, along with recovery time, in Appendix 1. 31. To date, chemical cleaning has been assessed on 3 full-scale sites. For 0.04 µm hollow fibre membranes operating at low to moderate flux (6 - 25L/(m2h)) intensive clean in place (CIP) and regular chemically enhanced backwash did not reduce LRV below typically observed process variability (5th percentile). However, when 0.4 µm flat sheet membranes, operated at high flux (30 L/(m2h)) underwent intensive CIP with NaOCl and Oxalic acid, a significant reduction in LRV occurred. Permeability change before and after cleaning was negligible for hollow fibre membranes, but increased 5 fold upon cleaning flat sheet membranes. A significant change in permeability from nominal conditions is considered to be a site specific indicator that membrane rejection may have reduced. 32. Membrane performance after 10 years was not significantly different to LRV documented for the same plant at 5 years operation. After membrane replacement, size exclusion improved resulting in an increase in retention of larger microorganisms. However, the new highly permeable membranes appeared to have lower virus rejection in situ than older fouled membranes. LRV for all indicators in situ was > 3.5 before and after replacement. 33. Pilot scale assessment of hazardous events demonstrated that plugging and shielding of damage to hollow fibre membranes could occur rapidly (< 15 min) and result in recovery of LRV to nominal values. Plugs were reversible and could be removed during backflush. High NaCl intrusion reduced virus rejection, believed to be due to dispersion of particles from sludge, but recovered within 2 days, upon washout from the reactor. Most other chemical shock loads induced severe fouling, that may have mitigated excess breakthrough as a result of reduced activated sludge performance.

Establishment of a default/indicative value for MBR (Appendix B)	34. A default or indicative LRV could be used to provide a basis for conservative accreditation of MBR systems where extensive validation testing is considered unfeasible. 35. Probability density functions (PDF) were fit to all data collected from literature (n > 1000 LRVs) and data from site sampling. In addition, an operating envelope from site sampling was specified corresponding to LRV. Also, the results from sampling of 2 sites to a total of 8 samples for cryptosporidium, giardia, enteroviruses, reoviruses and adenoviruses were reported (all > 4 LRV). 36. The 5th percentiles of resulting LRV PDFs were collated and the most conservative sets of viruses, bacteria and protozoa were rounded down to form the basis of default LRV. The following values are proposed as conservative indicative LRVs for MBR: o Virus: 1.5 o Bacteria: 4.0 o Protozoa 2.0 37. The 95th percentile of permeate turbidity for the corresponding operating envelope was 0.4 NTU. Hence, as long as permeate turbidity remains less than 0.4 NTU and MBRs are operated within the range of conditions specified in Table 8 of Appendix B, LRV is not likely to reduce below the values above.
Proposal of a Validation Protocol (Appendix A)	38. The findings of this research were translated into a validation protocol consistent with the template provided by the PDG. The resulting MBR protocol is a draft for discussion. 39. The proposed validation protocol is based extensively on the existing VDoH (2013) document. However, some alterations were made including a reduction in sampling requirement, consideration of eligibility for pre-validation and listing significant influencing parameters as a result of Bayesian analysis. 40. The reduction in sampling requirement was justified by suggesting samples should only be taken under the most conservative conditions, ie highest permeability (lowest fouling). In this way, the LRV determined during challenge testing should represent the worst case expected during operation. 41. Further improvements to the validation protocol may include a tiered approach, whereby a conservative default value can be claimed at the lowest tier.

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Research Report Executive Summary

To facilitate the development of informed and appropriate membrane bioreactor (MBR) validation guidelines, a thorough review of the scientific literature, existing MBR validation reports and the Victorian validation guidelines (VDoH 2013) was conducted. Where research gaps were identified, experimental work was planned accordingly. Over the duration of the NatVal project, SP1 has conducted over 180 site visits across 9 full scale MBRs (Section 6). Systematic case studies were conducted, in addition to collection of a large data set, representative of normal operation, in order to correlate MBR LRV with operating parameters.

From the literature review, an overview of removal mechanisms in MBR, the contributing factors to those removal mechanisms and a detailed assessment of reported MBR log removal value (LRV) were obtained. The key mechanisms include biodegradation, adsorption to membrane and biomass and size exclusion by the membrane. Systematic studies to establish mechanisms were based on removal of indicator species. There is a clear research gap regarding the correlation of fate and removal of indicator species with those of pathogens. Of the two most common virus indicators, somatic coliphage exhibited greater resistance to MBR treatment than FRNA bacteriophage (Sections 1 and 2).

LRVs extracted, from 33 published sources, were fit to normal distributions for the purpose of understanding treatment variability, as well as capability. The 5th percentile LRV for the most conservative set of virus, bacteria and protozoan indicators were 1.7, 3.5 and 3.5 respectively (Section 2).

Common membrane integrity monitoring techniques applicable to MBR were critically assessed including turbidity, particle counting and pressure decay testing. Turbidity and particle counting are applicable for online monitoring of membrane integrity for all configurations of MBR. However, the limit of detection of these techniques with regards to pathogen breakthrough remains unknown (Section 3).

Through contrasting elements of validation reports supplied by MBR stake holders with the process outlined in VDoH (2013), inconsistencies in state-based MBR validation requiring resolution prior to development of National Validation Guidelines were brought to light. These include the circumstances requiring a challenge test period for validation, the types of challenge organisms to be used (pathogens vs indicators) and the LRV accreditation received (Section 5).

MBRs can provide effective treatment for many wastewater contaminants including chemicals and microorganisms. Operational performances for the removal of these contaminants are most typically characterised under what are considered to be normal operating conditions. However, all MBR systems are continuously subjected to the risk of deviations in operating conditions during what have been termed 'hazardous events (HEs)'. HEs may include sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions. A qualitative assessment of relevant and likely HEs in MBRs was carried out (Section 4). In addition, impacts of a wide range of HEs on MBR performance were studied at lab-scale (Sections 11 and 12). Furthermore, investigation at full-scale MBRs was also conducted. Chemical shockloading was found to reduce LRV due to the bioreactor. LRV due to biopredation within the activated sludge contributed 20% of the overall process LRV Membrane integrity failure significantly reduced the LRV, but hollow fibre plugging and defect shielding resulted in rapid recovery within normal process variability in less than 24 hr. Appendix 1 contains an overview matrix of the impact of hazardous events on LRV and time to recovery.

Chemical cleaning of MBRs is a concerning event, as removal of the fouling layer may result in loss of containment of viruses that are smaller than the membrane pore size. Two chemical cleaning modes were investigated, a yearly clean in place (CIP) and a series of weekly chemically enhanced backwashes (CEB). CEB, with sodium hypochlorite, can result in large disinfectant quantities remaining in the permeate for up to 20 min. Immediately after CEB,

overall LRVs were increased due to presence of disinfectant. Recovery of LRV after CIP to within normal process variability occurred in less than 5 days (Section 8).

While experimental work has concluded, data analysis of the significant pool of LRVs and operational parameters collected in Section 6 is as yet incomplete. Section 7 details planned approaches for identification of influencing factors to ensure recommendations for the validation methodology are appropriate. Section 7 includes an update on the current Bayesian approach to modelling MBR LRV via operating parameters. Section 9 summarises a case study of membrane replacement after 10 years of operation. The findings from this study, suggested that even after significant ageing and damage LRV was not reduced more than 20% for larger microorganisms. The replacement of membranes with unfouled virgin modules exhibited lower virus removal than aged and damaged membranes. Biopredation appeared to be microorganism specific, but had a greater reduction at longer HRT. Section 10 details the aim and preliminary work of relating turbidity with LRV of MBR.

1 Review of Pathogen Removal Mechanisms in MBR

1.1 Introduction

The current state of knowledge surrounding pathogen removal mechanisms in MBR was reviewed. Factors that could influence pathogen removal were summarised from literature.

1.2 Mechanisms of removal

No single mechanism can be defined as the sole contributor to rejection of pathogens through MBR; A combination of size exclusion, adsorption and biodegradation, will be responsible. The properties of the pathogen will determine the dominant removal mechanism.

1.2.1 Size exclusion

Providing the integrity is sound, size exclusion is responsible for the rejection of any particle or pathogen larger than the pores of the membrane. Ultrafiltration (UF) and microfiltration (MF) membranes exhibit respective pore sizes, 0.002 – 0.05 µm, and > 0.05 µm (Judd 2011). The first fully operational European MBR plant, Porlock in the UK, averaged > 5 LRV for faecal coliforms when assayed over 6 years from 1998 – 2003. Virus LRV indicated by FRNA bacteriophage varied between 3 and 5 during the same period. The Porlock MBR is outfitted with 0.4 µm, flat sheet Kubota membranes (Severn 2003). Bacteria, such as faecal coliforms, are sized between 0.5 – 10 µm; while viruses, like FRNA bacteriophage, are typically 0.01 – 0.1 µm (Antony *et al.* 2011). In a full-scale study, the bacterial indicators enterococci, total coliforms and *E. coli* displayed LRVs > 5 log across the membrane. Removal from sewage to the mixed liquor was less than 0.5 log (van den Akker *et al.* 2012).

> *Size exclusion is the predominant mechanism for removal of organisms greater than the nominal pore size of the membrane.*

1.2.2 Adsorption

1.2.2.1 Adsorption to membrane

Adsorption of pathogens to the membrane, the cake layer (formed by the accumulated foulants on the membrane) or the biofilm (developing over prolonged filtration periods) has been cited as a contributing mechanism to explain appreciable rejection of pathogens, such as viruses (Ueda *et al.* 2000, Shang *et al.* 2005, Ottoson *et al.* 2006, Sima *et al.* 2011).
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. This peak adsorption was observed for a fouled microfiltration membrane operating at different fluxes (Farahbakhsh *et al.* 2004). As flux increased, coliphage retention increased to a maximum value. As flux further increased, it was hypothesized that the corresponding increase in shear rates through the biofilm resulted in release of coliphage captured by the biofilm resulting in an overall decrease in rejection. In another experiment with 0.22 µm membranes filtering directly poliovirus (0.028 – 0.030 µm) at lab scale, Madaeni *et al.* observed a peak rejection of virus 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). Coliphage f2 removal by 0.22 and 0.1 µm membranes was investigated (Zheng *et al.* 2006). Within the first 20 h, LRV for both membranes increased significantly, from less than 1 to above 2. This filtration time was considered insufficient for significant bio-fouling to occur (Drews 2010). It is more likely that adsorption to the reversible cake layer is responsible for the initial rapid increase in rejection.
Given appropriate time is allowed for its formation, biofilm may be responsible for enhanced virus rejection. At full scale, permeate norovirus concentrations varied sporadically and did not correspond with changes in influent and MLSS concentrations. A reversible bio-fouling

layer, that may be periodically disturbed, was proposed to explain this phenomenon (Sima *et al.* 2011).

1.2.2.2 Adsorption to biomass

The suspended solids component within the activated sludge presents another source of sites for adsorption of pathogens. In contrast to adsorption to the membrane, adsorption to MLSS is less reversible. As to control solids retention time (SRT), biomass is wasted as a constant rate, while new biomass grows; any pathogens adsorbed to the MLSS will be removed as excess sludge, potentially, before desorption can occur.
Adsorption of viruses to biomass and subsequent removal as waste activated sludge (WAS) was quantified through the use of molecular microbial analysis techniques on the solid portion of the WAS (Sima *et al.* 2011). Increasing Norovirus concentration in the WAS was observed and corresponded 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 3 log more Norovirus GII was present in settled solids when compared with supernatant of activated sludge from an MBR (Simmons *et al.* 2011); Similar observations for Norovirus, (i.e., 1 log 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 2 – 4 log 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). Quantitative Polymerase Chain Reaction (PCR) was used to assay the densities of pathogenic viruses in previous work. Infectivity studies were not conducted; hence, it is unclear whether once adsorbed to solids virus infectivity is hampered. Regardless, once adsorbed to biomass, the viruses can be rejected by size exclusion.

Adsorption of pathogenic viruses, smaller than the pore size, to MLSS permits rejection by the membrane and removal via the WAS.
Due to the use of QPCR for assay of pathogenic viruses, it is unclear if infectivity is reduced via adsorption.
Guidance is requested from Sub Project 2 regarding potential similarities, of adsorption behavior, with activated sludge.

1.2.3 Bio-predation

In activated sludge, bio-predation of pathogens by larger protozoa and metazoans is a mechanism for removal. Predation 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 behaviour of the activated sludge of an MBR system is likely to occur. CAS systems were attributed with an LRV for phage of 0.75 (Rose *et al.* 1996), at lab scale the biomass of an MBR was attributed with an LRV of 0.8 for MS2 phage (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 predation and adsorption to the membrane gel layer was responsible (Lv *et al.* 2006); unfortunately the relative effect of each bio-predation 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 role for biological degradation for these viruses (Kuo *et al.* 2010) (Simmons *et al.* 2011). 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). Cryptosporidium and Giardia have been observed to accumulate in the order of 1 log for a full scale MBR (Pettigrew *et al.* 2010).

Inactivation by bio-predation is significant at retarding bacterial accumulation in MBR.
For more biologically-resistant micro-organisms, viruses and protozoa, adsorption may be more significant.
Similarities in bio-predation of pathogens in MBR and CAS are expected. Further input from Sub Project 2 on this mechanism is requested.

1.3 Parameters effecting pathogen rejection

1.3.1 Membrane pore size

With direct implications for the pathogen removal mechanism of size exclusion (Section 1.2.1), membrane pore size would be expected to play a significant role; providing the membrane or module is not compromised. In MBRs, microfiltration (MF) and ultrafiltration (UF) membranes are commonly employed. Madaeni *et al*. compared the lab scale performance of clean MF (0.22 µm pore size) with UF (4 nm pore size), both challenged with solution of 10^4 /mL poliovirus (28 - 30 nm virus diameters). The MF filter approached a steady LRV of 1.6 – 1.7 after 3 h. The poliovirus could not be detected in the UF membrane permeate, resulting in an uncertain LRV of greater than 4 (Madaeni *et al.* 1995). During pilot scale evaluation, GE MBR UF with nominal membrane pore size 0.04 µm) were compared to Mitsubishi MBRs (MF nominal membrane pore size 0.4 µm) for the rejection of total coliphages native to primary effluent. The UF and MF pilots achieved LRVs of total coliphage of 4.0 - 5.5 and 3.0 – 5.0 respectively (DeCarolis *et al.* 2007). This result is not directly comparable with the work of Madaeni *et al*. as the distribution of sizes attributable to the native coliphages was not specified; hence, a quantitative ratio of coliphage size to membrane pore size is not available. The result of DeCarolis *et al*. does qualitatively permit the conclusion that variation of membrane pore size is not the principle determinant for virus rejection within the typical commercial range of MBR membrane pore sizes examined (0.04 – 0.4 µm). A similar study regarding the performance of commercial membranes with variation in pore size of 0.04 - 0.20 µm could find no significant difference in the rejection of seeded MS2 phage (Hirani *et al.* 2010). Lesjean suggests that there may be a slightly significant advantage of UF over MF membranes for virus rejection it is in the order of 0 - 1 log. Additionally, at full scale the overall module integrity, including seals & fibre potting interface, will play a significant role regardless of the membrane pore size (Lesjean *et al.* 2011).

Effective size exclusion of pathogens and consequent retention in the bioreactor, has prompted concerns of accumulation. If membrane integrity were lost, a greater health risk would result due to release of an increased concentration of pathogens. Utilising mass balance the approximate concentration factor of these pathogens is the SRT, assuming no biological predation. Identical findings regarding the densities of *E. coli* and sulphite reducing clostridia (SRC), from wastewater into the activated sludge, of the full scale MBRs have been reported. A negligible difference in the concentration of *E. coli* in the wastewater relative to the activated sludge was observed. Both studies observed an increase in SRC from wastewater to activated sludge of approximately 1 log (Marti *et al.* 2011) (van den Akker *et al.* 2012). This can be partially explained by the propensity of SRC to form spores, one of the reasons it is chosen as an indicator for cryptosporidium. The spores display increased resistance to biological predation. This example highlights that different mechanisms will be responsible for removal, depending on the nature of the pathogen.

> *Membrane pore size between 0.04 and 0.4 um does not appear to significantly affect virus rejection under steady state conditions.*
> *Accumulation of pathogen in biomass can occur, but is proportional and limited by the SRT. Accumulation does not appear to occur for organisms susceptible to bio-predation.*

1.3.2 Membrane material

Commercial MBR membranes are generally made from polymers, including polyethersulfone (PES), polysulfone (PS), polyvinylidene difluoride (PVDF), poyacrylonitrile (PAN), polypropylene (PP), polyethylene (PE) or polytetrafluoroethylene (PTFE). These polymers are often modified with additive copolymers (e.g., polyvinyl pyrrolidone (PVP)) during manufacture to increase final membrane hydrophilicity, which improves membrane permeability and can reduce the tendency to some types of fouling (Judd 2011). . The polymers used for MBR membranes exhibit relative advantages and disadvantages in terms of chemical resistance, strength, hydrophilicity and cost that dictates preference of a manufacturer to one base polymer type over another that is not relevant to this study. Relative hydrophobicity of the membrane will affect the capacity of the membrane to adsorb pathogens. When comparing the rejection of MS2 phage at neutral pH with hydrophobic and

hydrophilic membranes, it was noted that rejection of the hydrophilic membrane was lower. This was attributed to a higher adsorption tendency to a hydrophobic than a hydrophilic membrane (van Voorthuizen *et al.* 2001). The significance of the relative hydrophobicity of differing membrane materials and its effect on virus rejection is questionable at pilot and full scale MBR operation, likely due to the many other uncontrollable variables at this level. In a pilot study consisting of various commercial membranes of PVDF, PE and chlorinated PE, no significant difference in virus removal could be attributed to membrane material (DeCarolis *et al.* 2007).

Membrane material is not a significant factor influencing pathogen rejection at full scale.

1.3.3 Solid retention time and MLSS

It is generally assumed that high concentration of MLSS would result in greater level of depositing on the membrane and consequently in higher rejection. The effect of MLSS at (6, 8 and 10 g/L) on MS2 phage removal by a 0.4 µm membrane MBR was investigated (Shang *et al.* 2005). After 14 days, no significant contribution of the increased MLSS concentration was observed, with the biofilm rejection stabilising at an approximate LRV of 2.5. Interestingly, the MLSS concentration of 6 g/L took only 4 days to achieve the LRV of 2.5, while the MLSS of 10 g/L did not exceed an LRV of 2 until the 8th day of filtration. These results presented opposite trends to the previously mentioned assumption. The authors noted that the amount of and composition of extracellular polymeric substances (EPS) were more likely related with the formation of the biofilm and that occurrence of EPS was dependent on food to microorganism ratio (F/M ratios) (Shang *et al.* 2005). In a system with constant feed concentration, increase in MLSS results in reduced F/M ratio. In the same study, contradiction when investigating an increase in SRT at constant MLSS was also noted. The longer SRT (200 days) corresponding to a low F/M ratio resulted in higher phage removal (1 LRV) than the lower SRT (50 days) corresponding with a high F/M ratio (Shang *et al.* 2005). This contradiction presents potential further investigation into finding an optimum balance, between the interrelated SRT, MLSS and F/M ratio, that is relevant to real operating conditions; an SRT of 50 - 200 days being far longer than a typical MBR operation. A larger MLSS, 6 – 8 g/L (due to increased SRT), was observed to correspond with higher concentrations of SRC and FRNA bacteriophage in the activated sludge of an MBR (van den Akker *et al.* 2014).

The overall significance of SRT and MLSS is unclear. For smaller organisms, a higher MLSS may present more adsorption sites.
A longer SRT is expected to result in greater accumulation of organisms (rejected by size exclusion and resistant to biodegradation); hence, a higher concentration challenging the membrane.
F/M Ratio may effect formation of a bio-fouling layer on the membrane that enhances virus rejection.

1.3.4 pH

pH has been shown to affect both the zeta potential and hydrodynamic radii of viral indicators T4 coliphage and MS2 phage. Arkhangelsky at al. reported changes in hydrodynamic radii of MS2 from 15 – 170 nm over a pH range of 3 – 10, decreasing below 20 nm from pH 5 - 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 vary between 60 – 140 nm for the pH range of 3 – 10 (Arkhangelsky *et al.* 2008). Early work on MF membranes in clean water indicated higher rejection of virus indicators MS2, exceeding 80% at pH4, and T4 coliphage at low pH. 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 *et al.* 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 virus IEP (pH 3 - 5(Michen *et al.* 2010)), promoting virus to virus interaction, could be considered extreme events.

Extreme pH changes may affect particle to particle and particle to sludge adsorption. Decrease in pH below the pathogen IEP may improve rejection.

1.3.5 Fouling

Fouling can be defined as "the inevitable coverage of the membrane surface (external and internal) by deposits which adsorb or accumulate during operation" (Drews 2010). This complex phenomenon can be characterized by a sequence of different stages, occurring in MBRs at various rates and time scales. The different stages of fouling can be defined according to the cleaning strategy necessary to remove the corresponding fouling phenomena as follows. Reversible fouling (cake filtration) occurs within 10 min of MBR operation with an associated trans-membrane pressure (TMP) increase of 0.1 – 1 mbar/min. Reversible fouling refers to fouling that can be removed by physical means such as backflushing, aeration or relaxation. Residual fouling occurs within 1 – 2 weeks of operation at a rate of 0.01 – 0.1 mbar/min. Residual fouling requires maintenance cleans, such as hosing or chemically assisted backwash (Brepols *et al.* 2008). Irreversible fouling was defined as fouling requiring 'main cleans' such as removal of membranes and soaking in cleaning solution. The irreversible fouling rate and time frame were 0.001 – 0.01 mbar/min and 6 – 12 months respectively. Finally, the term irrecoverable fouling was used to describe the long term membrane permeability loss that could not be recovered and corresponded to a fouling rate of 0.0001 - 0.001 mbar/min and a time frame of greater than 1 year (Drews 2010).

If the mechanism for virus removal via adsorption to filter cake and biofilm was a significant contributor, one would expect to observe a gradual rise in virus rejection (in the order of weeks) from start-up with a clean membrane. A physical cleaning event would result in a lower LRV, followed by further drop in virus rejection following a chemical cleaning event; due to the purpose of these cleaning regimes at mitigation of reversible and residual-irreversible fouling respectively. Experiments were conducted challenging a lab scale MBR (PE membrane with pore size 0.4 µm) with MS2 phage (24 nm). After 9 days, the effect of the biofilm was not significantly improved relative to the clean membrane on startup (LRV = 0.3), while, after 21 days, the rejection of the membrane increased to an LRV of 2.1. The authors also noted that application of chemically assisted backwash with 600 mg/L sodium hypochlorite (NaOCl) reduced the virus rejection to the level of the membrane at start up, indicating the destruction of the biofilm (Shang *et al.* 2005). In a different study, 0.22 µm PVDF MBR membrane with a previously formed biofilm and cake layer was removed from the biomass and challenged with T4 coliphage (mean diameter 0.08 – 0.12 µm) in tap water to assess the effect of the accumulated fouling layers on virus removal(Xiang *et al.* 2005). The initial T4 coliphage LRV was approximately 6. The membrane was then rinsed with water to destroy the cake layer. Following 'soft' destruction of the cake layer, the LRV dropped to 2 – 3. The membrane was then chemically cleaned by soaking for 12 h in 0.7% sodium hydroxide (NaOH), followed by 2% NaOCl to remove the biofilm. The LRV following biofilm destruction was 1 – 2. In parallel, the same approach was applied to a PP MBR membrane with pore size 0.1 µm (i.e. approximately the same size as the T4 virus). The rejection change was negligible with the PP membrane indicating the dominance of size exclusion, when membrane pore size approaches pathogen pore size (Xiang *et al.* 2005).

In a pilot scale experiment comparing various commercial MBR systems, backwashing events, of a 0.08 – 0.2 µm US Filter MBR, corresponded with short-term decrease in rejection of total coliforms. The decrease in rejection of total coliforms was not seen in a parallel pilot, using Mitsubishi Sterapore membranes 0.4 – 0.5 µm, that employed relaxation in place of backwashing. Comparative loss of rejection, was attributed to destruction of the cake fouling layer and coliform growth in the backwash tank, allowing intrusion of coliforms into the permeate (DeCarolis *et al.* 2007). These studies highlight the significant contribution of the adsorption to biofilm and cake filtration mechanism to enhance pathogen rejection when the pathogen is smaller than the membrane pore size. Furthermore, results suggest that a cake fouling layer may shield integrity defects larger than the nominal membrane pore size.

Enhanced rejection of viruses due to formation of a biofilm is plausible, but the biofilm takes time to form.
Initial change in rejection may be due to more rapid formation of the reversible cake layer.

1.3.6 Membrane cleaning

MBR cleaning practices present potential impacts to LRV through removal of the fouling layer (Section 0), and alteration of the intrinsic properties of the membrane.
Puspitasari *et al*. investigated the chemical alteration of PVDF membranes aged in 1% NaOCl solution for up to 21 weeks. Results indicated that the apparent mean pore diameter increased from 0.19 to 0.21 µm after the first week then reduced to 0.16 µm in the second week and recovered to a stable average of 0.21 µm by week 11. Additional measurements were made of relative membrane hydrophobicity during the same ageing experiment. The contact angle of the virgin membrane was 29°, after 2 weeks aging the contact angle had increased to 65° indicating an increase in relative membrane hydrophobicity. Subsequent decrease was then observed and in the 21st week of ageing the contact angle had recovered to 8°, indicating a reduction in relative hydrophobicity compared to the virgin membrane (Puspitasari *et al.* 2010). Long term increase in average pore size will decrease the size exclusion ability of the membrane; however, the changes reported in this study can be considered marginal, given the harsh ageing condition the membranes were subjected to in that particular study. In another study (Wang *et al.* 2010), the effect of cyclical cleaning and operation of PVDF membranes used in MBRs were studied. The contact angle was initially hydrophobic (above 90°C) and displayed lower contact angles with subsequent cleanings, indicating the same change to a less hydrophobic state. NaOCl treatment on PES ultrafiltration membranes resulted in a decreasing contact angle with increasing NaOCl contact time (Arkhangelsky *et al.* 2007). As mentioned previously, fouling layer development and capacity of the membrane for pathogen adsorption will be reduced via a change in relative hydrophobicity; however, the significance of this effect on full scale operation is yet to be observed.

The tensile strength at break, elongation at break and Young's modulus values of the PES membrane showed decline with increasing NaOCl contact time (Arkhangelsky *et al.* 2007). This result confirmed that mechanical damage resulted upon exposure of membranes to cleaning chemicals. In a separate study, impact of chlorine exposure on weld strength of the membrane/module connection, widely accepted as one of the weakest integrity points of MBR membrane modules (Judd 2011), was investigated. A correlation was determined such that, after 1.3 kg of NaOCl was dosed per module the welding strength would be below the minimum for a virgin cartridge, set by the manufacturer; indicating a higher likelihood of integrity failure (Ayala *et al.* 2011).

> *After long term operation, cleaning chemicals used to clean the membrane are expected to alter membrane material properties, but this effect on LRV has not been quantified in full scale systems.*
> *Long term chemical exposure may increase likelihood of integrity failure due to embrittlement of module materials and interfaces.*

1.3.7 Membrane ageing and lifetime

MBR technology has not yet reached maturity, as the typical operation lifetime for membranes before failure has not been defined. So far, membrane and MBR suppliers offer specific lifetime guarantees in the order of 3 – 8 years (Le-Clech 2010). Recently, predictions of membrane life have been made via different methods. From correlation of total membrane through-put with mechanical stability loss of the membrane-module bond, a lifetime of 6.4 years at a mean flux of 20 L m^{-2} h^{-1}, or 8.5 years at 15 L m^{-2} h^{-1} was proposed (Ayala *et al.* 2011). Through rigorous assessment of North American sales data for Zenon MBR membranes and classification of the type of sale (replacement or new), an empirical model yielded a membrane/module life of 8 years; it was also stated that most failures were attributed to early generation module issues, which were no longer observed with the later generation; hence, the 8 year estimate was likely conservative (Cote *et al.* 2012). Through extrapolation of permeability (i.e., production capacity) decline to an unacceptable level, a lifetime estimate of 8 – 10 years was made. The model was based on observation of GE Zenon membranes, for 9 years, at one full scale facility (Fenu *et al.* 2012).

So far, membrane life estimates have been ultimately based on noticeably gross integrity failure, loss of mechanical strength, or production decline. Trend of MBR permeate microbial

quality over long term operation is an area where little rigorous published data exists. In a 10 year study of the Porlock STP MBR from 1998 – 2007, average LRVs for Faecal coliforms and F + coliphage were reported as > 5 and > 4 respectively; however, no mention of a trend in effluent quality or LRV was mentioned (Nishimori *et al.* 2010). A rigorous assessment of the LRV for a range of microorganisms was performed on the MBR at North Head Sewage treatment plant Manly in February and March 2010, which had been operating for 5 years at the time of analysis. Performance during this period appeared typical of other pilot assessments with new MBRs. Reported LRVs were 5 – 7 for *E. coli* and 4 – 6 for F specific RNA bacteriophage (Pettigrew *et al.* 2010). Unfortunately, the lack of any initial pathogen removal performance data prohibited assessment of any change in quality.

MBR membrane life is predicted in excess of 8 years. No data has been presented regarding the potential impact of lifetime on LRV.

1.4 Conclusions

Removal mechanisms for MBR were reviewed. The key mechanisms include biodegradation, adsorption to membrane and biomass and size exclusion by the membrane. Various factors may influence the effectiveness of these mechanisms and were critically reported. Changes in pH may affect adsorption characteristics but for indicators the range of pH (pH 3 – 5) needed is out of the typical range of MBR operation. Literature investigations regarding removal mechanisms, were based on the use of indicator species. Therefore, there is a clear research gap regarding correlation of fate and removal of indicator species with pathogens.

Only nine studies reporting appropriate LRV performances for pathogens have been found. Due to the limitations of QPCR in assessing pathogen viability, the relative role of the bioreactor (adsorption and biodegradation) in the deactivation of pathogens has not been established. Measurement of pathogenic species has further been hampered by the extreme variability of concentrations in wastewater. However, a common trend of pathogenic viruses preferentially associating with suspended solids in the bioreactor was observed.

2 Pathogen Removal Performances in MBR

2.1 MBR removal performance data

Data fitting was employed to summarise the magnitude and variability of LRVs taken from validation reports and literature. Advice on the data analysis methods used, the adequacy of a normal distribution for description of LRV, and the application of the results for pre validation risk assessment, is requested from Sub Project 5.

2.1.1 Collation of LRV data

LRV (and where possible influent, mixed liquor and permeate concentrations) for indicator microorganisms and pathogens as well as operational metadata have been collected and stored in a format to allow statistical comparison. Data was collected from published literature (33 sources) and validation reports (2 validation data sets). Of the literature surveyed, only 9 sources contained data for pathogens the balance measuring indicator species. Values have been collated either from values presented in tables or via linear interpolation of data points from graphs. References to papers where data was extracted are listed below: (van den Akker *et al.* , Chiemcharisri *et al.* 1992, Dowd *et al.* 1998, Gander *et al.* 2000, Ueda *et al.* 2000, Chang *et al.* 2001, Gantzer *et al.* 2001, Mooijman *et al.* 2001, Severn 2003, Oota *et al.* 2005, Shang *et al.* 2005, Xiang *et al.* 2005, Friedler *et al.* 2006, Hirani *et al.* 2006, Lv *et al.* 2006, Ottoson *et al.* 2006, Zheng *et al.* 2006, DeCarolis *et al.* 2007, Silva *et al.* 2007, Tam *et al.* 2007, Zhang *et al.* 2007, Hirani *et al.* 2010, Kuo *et al.* 2010, Michen *et al.* 2010, Nishimori *et al.* 2010, Pettigrew *et al.* 2010, Wu *et al.* 2010, Zanetti *et al.* 2010, Marti *et al.* 2011, Mosqueda-Jimenez *et al.* 2011, Sima *et al.* 2011, Simmons *et al.* 2011, Francy *et al.* 2012, Hirani *et al.* 2012, Keskes *et al.* 2012, Trinh *et al.* 2012, van den Akker *et al.* 2012, De Luca *et al.* 2013, Hirani *et al.* 2013).

So far a total of 684, 597 and 26 LRV were analysed for indicators or pathogens from bacteria, virus and protozoan groups respectively.

2.1.2 Data analysis

To approximate a probability distribution function (PDF) for MBR LRV, data sorting and Monte Carlo simulation was performed according to the method suggested previously (Khan 2010). Due to constraints regarding the nature of the data reported, three different modified methods were used to process LRV data into a total of 48 normal PDFs. The fitting methods along with associated constraints were summarised below. Review of the fitting methods and resultant normal distribution parameters is requested from Sub Project 5.

2.1.2.1 Fitting Method 1

2.1.2.1.1.1 Data Requirements

- Raw concentrations (Feed, Permeate and Activated Sludge)
- Knowledge of the detection limit for each set
- At least 3 values in each set to be used above the detection limit.

2.1.2.1.1.2 Method

Each independent set is sorted from lowest to highest value.
Each point in the set is assigned a 'p-value' with the blom formula (Equation 1)

$$p = \frac{(i-0.375)}{(n+0.25)} \qquad \text{Equation 1}$$

Where: p is the resulting p value (between 0 – 1), i is the order of the point in the data set (eg the first point is i = 1, second point is i = 2) and, n is the total number of points in the set.
All points above the limit of detection and their p values are highlighted and a log normal distribution is fitted using @risk software (Palisade 2013) (Figure 1).

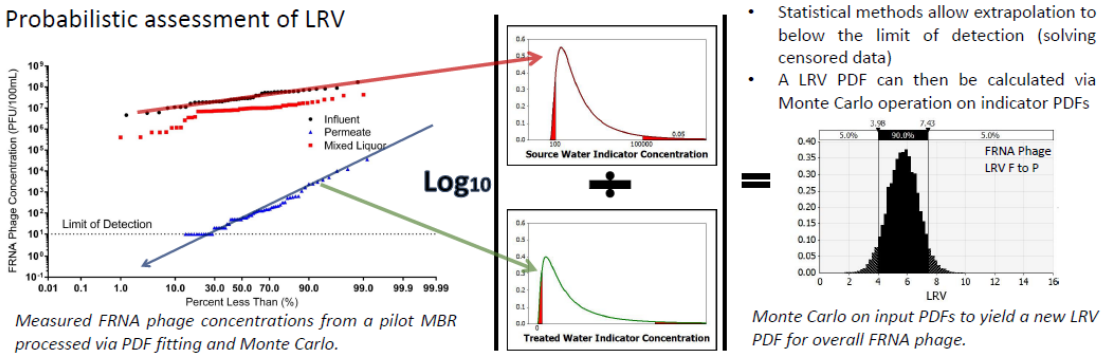

Figure 1 - Illustration of Method 1. Note 30% of permeate FRNA phage were below the detection limit, hence only the higher 70% have been used in @risk to create the intermediate distribution.

Table 1 - Advantages and disadvantages of fitting Method 1.

Advantages	Disadvantages
Efficiently deals with censored LRV values e.g. > LRV	Appears to yield broader (higher standard deviation) distribution than a paired LRV analysis of the same data set.
	Data often not provided as raw concentrations
	Cannot be used to combine across different site data due to step changes in feed distribution

2.1.2.2 Fitting Method 2

2.1.2.2.1.1 Data Requirements

- LRV data set
- Knowledge of whether or not the paired LRV is the true value (detected in the permeate) or censored e.g > LRV
- At least 3 of the lowest LRV must be uncensored

2.1.2.2.1.2 Method

LRV are sorted from lowest to highest and assigned a p value similarly to the concentration data points in method 1.
All of the lower LRV values and their p values, before the first non-detect are fitted to a normal distribution in @risk (Figure 2).

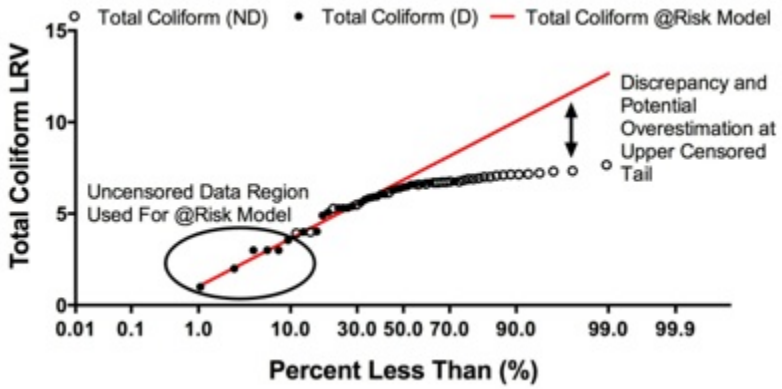

Figure 2- Method 2 fitting procedure. Note feed water changes combined with removal performance variation means that there may be censored LRVs dispersed among the set of absolute LRV.

Table 2 - Advantages and disadvantages of fitting Method 2.

Advantages	Disadvantages
Provides an LRV distribution calibrated to conservative lower values.	No way of assessing if performance is over estimated in upper censored region.
Can be used to combine sets of LRV from different sources/sites (performance not dependant on feed distribution).	For larger organisms (bacteria and protozoa) with MBR often there are not > 3 uncensored data points at the lower end of the distribution.

2.1.2.3 Fitting Method 3

2.1.2.3.1.1 Data Requirements

- LRV data set

2.1.2.3.1.2 Method

LRV are sorted from lowest to highest and assigned a p value similarly to the concentration data points in method 1.
All of the LRV values and their p values are fitted to a normal distribution in @risk (Figure 3).
For comparison the same data set for Figure 2 has been utilised.

Figure 3 – Method 3 Fitting Procedure. Note the LRV although now based on more data points exhibits a higher 5th percentile, similar average and lower higher percentile performance.

Theoretically method 3 should result in a conservative distribution as it assumes the upper bounded LRVs are a true value.

Table 3 - Advantages and disadvantages of fitting method 3.

Advantages	Disadvantages
Provides an LRV distribution calibrated to a large number of reported values.	Possibility of over estimating lower percentile performance.
Can be used to combine sets of LRV from different sources/sites (performance not dependant on feed distribution).	Ignores the effect of censored data.
	Non normality (of assuming a censored value is true) can result in curvature and poor model fit.

2.1.3 Data reporting

Data regarding pathogenic viruses, indicator viruses, bacteria and protozoa were grouped into 4 tables. For pathogen groups of interest, the normal distribution fit parameters mean (μ) and standard deviation (σ), as well as the 5^{th} and 95^{th} percentile LRV of the model and the number of LRV (or concentration) data input were included in Tables 4 to 7. For fitting Methods 2 and 3, the ratio of LRVs that were detected in the permeate (d) over the total number of LRVs (n) was reported. For fitting method 1 the d/n ratio was represented for as n/d F, n/d ML and n/d P for feed, mixed liquor and permeate concentrations respectively. Specific references containing the source data for normal distribution models were summarized in Table 8 in the appendix.

In cases where fitting Method 2 was not applicable, it was due to the presence of non-detects within at least the lowest 3 LRV reported. Where Method 1was not applicable, the data has been combined across different sites or sources, or no concentration data was presented, only LRV. Method 3 was only considered non applicable (N/A) when all permeate were detected, hence fitting Method 3 was identical to Method 2. Where multiple sources have been used the number in brackets represents the number of independent data sources e.g. All Sources (3) was compiled from 3 different sources.

2.2 Removal of pathogens and indicators by MBR

The data presented in Tables 4 – 7 were summarised as mean and standard deviation to permit quantitative microbial risk assessment (QMRA) of MBR processes either, deterministically, or probabilistically. In this format, both the LRV value and variability are expressed.

Table 4 - Normal distribution parameters for MBR pathogenic virus LRV data.

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
Adenovirus (species not specified)	All Sources (4)	N/A					N/A					3.37	1.14	1.49	5.25	16/24
	Source 1	3.87	1.36	1.64	6.08	11/11 F 5/11P	3.39	0.77	2.13	4.66	5/11	3.71	0.97	2.11	5.30	5/11
	Source 2	2.77	0.92	1.27	4.29	8/8 F, ML & P	2.74	0.34	2.18	3.30	8/8	N/A				
Adenovirus species A	Source 2	1.98	1.16	0.05	3.88	6/8 F 7/8 ML 8/8 P	2.33	0.33	1.79	2.87	6/6	N/A				
Adenovirus species C	Source 2	2.22	0.41	1.56	2.90	8/8 F, ML & P	2.23	0.31	1.73	2.73	8/8	N/A				
Adenovirus species F	Source 2	3.83	1.62	1.13	6.49	7/8 F 8/8 ML 8/8 P	3.47	0.71	2.30	4.64	4/7	3.41	0.64	2.35	4.47	4/7
Enteric viruses (by infectivity)	Source 1	N/A					N/A					2.55	0.48	1.76	3.33	0/8
Enterovirus	All Sources (5)	N/A					N/A					3.52	1.36	1.28	5.75	5/16
Norovirus (genogroup not specified)	All Sources (2)	N/A					N/A					4.45	1.92	1.23	7.61	8/15
Norovirus GI	All Sources (3)	N/A					N/A					3.33	1.65	0.62	6.05	12/30
	Source 3	4.28	1.61	1.61	6.90	14/14 F 13/14 ML 7/14 P	N/A					4.31	1.15	2.42	6.20	7/14
Norovirus GII	All Sources (2)	N/A					N/A					4.51	1.27	2.42	6.60	4/23
	Source 3	4.57	1.88	1.50	7.65	13/15F 15/15ML 4/15 P	N/A					4.49	1.84	1.48	7.51	4/15
Norovirus GIV	Source 3	N/A					N/A					3.22	0.64	2.18	4.27	0/6
Sapovirus	Source 3	N/A					N/A					2.62	0.76	1.36	3.87	1/16

Table 5 - Normal distribution parameters for MBR indicator virus LRV data.

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
Bacteroides fragilis phage	All Sources (3)	N/A					N/A					3.66	0.60	2.67	4.64	0/9
FRNA bacteriophage	All Sources (8)	N/A					4.93	1.23	2.91	6.95	110/130	4.58	0.97	2.99	6.17	110/130
	Source 4, FS 5 Year Study	5.93	1.61	3.34	8.61	77/77 F 12/77 P	N/A					4.61	0.92	3.09	6.12	12/77
	Source 5, HF At 5 Years	5.71	0.92	4.20	7.22	6/6 F 3/6 P	N/A					5.29	0.53	4.42	6.16	3/6 LRV
MS2 bacteriophage	Source 6	N/A					N/A					4.14	1.45	1.75	6.52	10/48
Qβ bacteriophage	Source 7 (0.03 – 0.1 μm)	5.50	1.07	3.75	7.26	9/9 F 9/9 P	5.57	1.31	3.42	7.72	9/9	N/A				
Somatic coliphage	All Sources (9)	N/A					3.58	0.85	2.18	4.99	84/139	3.43	0.77	2.17	4.69	84/139
	Flat Sheet 0.4 μm (6)	N/A					3.33	0.54	2.45	4.21	27/28	3.46	0.77	2.20	4.72	27/28
	Hollow Fibre (0.04 μm) (3)	N/A					3.58	0.82	2.23	4.94	33/84	3.65	0.61	2.65	4.66	33/84
T4 coliphage	Sources (8, 9) 0.22 μm	6.13	1.77	3.23	9.03	31/31 F 21/31 P	7.44	3.18	2.20	12.67	21/31	5.99	0.62	4.97	7.02	21/31
	Sources (8, 9) 0.10 μm	5.87	1.16	3.98	7.79	17/17 F 7/17 P	5.97	1.43	3.62	8.32	7/17	5.83	1.03	4.13	7.53	7/17
Total coliphage	All Sources (2)	N/A					4.42	0.80	3.11	5.73	15/41	4.39	0.36	3.80	4.97	15/41
	Source 10 0.04 μm HF	N/A					4.53	0.13	4.32	4.73	7/12	4.59	0.24	4.20	4.98	7/12
	Source 10 0.4 μm HF	4.33	1.48	1.91	6.76	13/13 F 7/13 P	3.81	0.61	2.80	4.81	7/13	4.05	0.83	2.69	5.41	7/13
All Virus	Indicators & Pathogens	N/A					6.65	2.56	2.43	10.85	334/597	3.95	1.35	1.73	6.16	334/597

Table 6 - Normal distribution parameters for MBR bacteria LRV data.

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
E. coli	All Sources (12)	N/A					N/A					5.87	0.71	4.71	7.04	40/95
	Source 11 incl. readings around cleaning	5.12	0.97	3.51	6.72	23/23 F 15/15 ML 15/18 P	N/A					N/A				
	Source 5 MBR at 5 years	5.78	0.37	5.17	6.38	6/6 F 6/6 P	5.71	0.26	5.28	6.14	6/6	N/A				
Enterococci	All Sources (5)	N/A					5.83	0.78	4.55	7.12	12/17	5.81	0.72	4.62	6.99	12/17
	Source 2 FS 0.4 μm	6.29	0.89	4.82	7.75	11/11 F 7/11 P	6.30	1.06	4.56	8.04	7/11	6.08	0.70	4.93	7.23	7/11
Faecal Coliforms	All Sources (10)	N/A					5.49	0.69	4.35	6.62	196/361	6.01	0.72	4.83	7.19	196/361
	Source 4 FS 5 years operation	5.89	0.50	5.05	6.71	143/143F 113/143P	6.03	0.67	4.93	7.13	113/143	5.97	0.62	4.95	6.98	113/143
	Source 4 FS 2.5 years operation	5.90	0.68	4.77	7.02	77/77 F 50/77 P	6.11	0.97	4.50	7.71	50/77	5.98	0.68	4.86	7.10	50/77
	Source 6	N/A					N/A					5.89	0.57	4.96	6.83	10/42
Total Coliforms	All Sources (11)	N/A					6.92	1.96	3.69	10.1	107/261	6.17	1.21	4.17	8.16	107/261
	Source 10 HF 0.08 μm	5.21	1.03	3.50	6.90	28/28 F 28/28 P	5.21	1.06	3.46	6.95	28/28	N/A				
	Source 10 HF 0.40 μm	6.62	0.64	5.57	7.67	13/13 F 9/13 P	6.59	0.45	5.86	7.33	9/13	6.61	0.53	5.74	7.48	9/13
	Source 11 incl. readings around cleaning	5.62	0.76	4.38	6.87	24/24 F 17/17 ML 13/14 P	N/A					N/A				
All Bacteria	Indicators and Pathogens	N/A					8.09	2.12	4.60	11.6	355/684	6.02	0.84	4.64	7.41	355/684

Table 7 - Normal distribution parameters for MBR indicator protozoa LRV data.

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
Clostridium perfringens	All Sources (5)	N/A					N/A					4.61	0.41	3.95	5.28	20/21
	Sources 11,13,14 incl. readings around cleaning 0.1 - 0.2 µm HF	N/A					4.63	0.45	3.88	5.37	10/10	N/A				
	Source 12 FS 0.4 µm Pilot	N/A					4.65	0.34	4.10	5.21	10/10	N/A				
	Source 11 incl. readings around cleaning	5.16	0.59	4.20	6.11	20/20 F 17/17 ML 14/14 P	N/A					N/A				
All Protozoa	Indicators and Pathogens (mostly clostridia)	N/A					N/A					4.49	0.60	3.50	5.48	21/26

Table 8 - Specific references for normal distribution data.

Source Number	Reference
1	Francy, D. S., E. A. Stelzer, R. N. Bushon, A. M. G. Brady, A. G. Williston, K. R. Riddell, M. A. Borchardt, S. K. Spencer and T. M. Gellner (2012). "Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters." Water Research 46(13): 4164-4178.
2	Kuo, D. H., F. J. Simmons, S. Blair, E. Hart, J. B. Rose and I. Xagoraraki (2010). "Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater." Water Res 44(5): 1520-1530.
3	Sima, L. C., J. Schaeffer, J.-C. L. Saux, S. Panaudeau, M. Elimelech and F. S. L. Guyader (2011). "Calicivirus removal in a membrane bioreactor wastewater treatment plant." Applied and Environmental Microbiology 77(15): 5170-5177.
4	Severn, R. (2003). "Long Term Operating Experience with Submerged Plate MBRs." Filtration and Separation 40(7): 28-31.
5	Pettigrew, L., M. Angles and N. Nelson (2010). "Pathogen removal by a membrane bioreactor." Journal of the Australian Water Association 37(6): 44-51.
6	(Hirani *et al.* 2012) Hirani, Z. M., J. F. DeCarolis, G. Lehman, S. S. Adham and J. G. Jacangelo (2012). "Occurence and removal of microbial indicators from municipal wastewaters by nine different MBR systems." Water Science & Technology 66(4): 865 - 871
7	Chiemcharisri, C., Y. K. Wong, T. Urase and K. Yamamoto (1992). "Organic Stabilisation and Nitrogen Removal in a Membrane Seperation Bioreactor for Domestic Wastewater Treatment." Water Science & Technology 25(10): 231 - 240
8	Xiang, Z., L. Wenzhou, Y. Min and L. Junxin (2005). "Evaluation of virus removal in MBR using coliphages T4." Chinese Science Bulletin 50(9): 862-867.
9	Lv, W., X. Zheng, M. Yang, Y. Zhang, Y. Liu and J. Liu (2006). "Virus removal performance and mechanism of a submerged membrane bioreactor." Process Biochemistry 41(2): 299-304.
10	DeCarolis, J. F. and S. Adham (2007). "Performance Investigation of Membrane Bioreactor Systems During Municipal Wastewater Reclamation." Water Environment Research 79(13): 2536-2550.
11	van den Akker, B., T. Trinh, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan (2014). "Validation of a full-scale membrane bioreactor and the impact of membrane cleaning on the removal of microbial indicators." Bioresoure Technology(0).
12	Marti, E., H. Monclús, J. Jofre, I. Rodriguez-Roda, J. Comas and J. L. Balcázar (2011). "Removal of microbial indicators from municipal wastewater by a membrane bioreactor (MBR)." Bioresource Technology 102(8): 5004-5009.
13	Trinh, T., B. v. d. Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan (2012). "Removal of endocrine disrupting chemicals and microbial indicators by a decentralised membrane bioreactor for water reuse." Journal of Water Reuse and Desalination 2(2): 67 - 73.
14	Validation of a full-scale Membrane Bioreactor for water recycling: Characterising process variability. Ozwater 2012. Sydney, Australia.

Pathogenic virus data surveyed included adenovirus (AdV), enterovirus (EV), norovirus (NoV) and sapovirus (SV) with 5th percentile LRVs > 1.5, 1.3, 1.2 and 1.4 respectively. Readings above the detection limit in the permeate occurred for 66% (AdV), 31%(EV), 53%(NoV) and 6%(SV) of samples. Somatic coliphage and FRNA bacteriophage 5th percentile LRV, from the literature review, were 2.2 and 2.9. Somatic coliphage and FRNA bacteriophage were detected in the permeate in 60% and 85% of cases, from a data set 10 times larger than the pathogenic LRV data. More importantly, the lower LRVs for FRNA and SC were not censored with permeate non-detects, meaning, their extrapolation theoretically approaches a true value when compared to the limited case of the pathogenic viruses; the difference between method 2 and method 3 for data fitting.

The pathogenic virus data (Table 4) exhibited high standard deviations in a majority of distributions fitted by Method 1. This is likely due to the seasonal variability of pathogens in wastewater producing

very significant concentration ranges when analysed over long periods (6 months – 1 year). Additionally, almost every source exhibited different detection limits as QPCR methods evolved from 2005 – 2012. When compared to indicator viruses, the pathogenic species data set is far smaller (one tenth of the values), presenting a further source of error.

Of the indicator viruses MS2 bacteriophage (a subset of FRNA bacteriophages), and somatic coliphage demonstrated the highest resistance with 5th percentile LRVs of 1.8 and 2.2 respectively. Indigenous coliphage (reported as total coliphage) and somatic coliphage exhibited slightly higher mean LRV for smaller pore size membranes 0.04 µm (hollow fibre) compared to 0.40 µm (hollow fibre and flat sheet). However, the standard deviation of the smaller pore size membrane LRV data set was larger; to the point where 5th percentile LRVs were not significantly different (Table 5). Pathogenic and indicator virus data was combined as a whole data set (334 detected/597 total values) and yielded a 5th percentile LRV of 1.7.

For three of the lowest distributions, bacterial indicators were removed > 3.5 log, 95% of the time. Mean removal for all bacterial indicators ranged from 5 – 7 log units (Table 6). The combined set of all bacterial indicators (354 detected /684 total values) yielded a 5th percentile LRV of 4.6.

The protozoan indicator set comprised mainly of *Clostridium perfringens* (Table 7) but included, three cryptosporidium and two giardia LRV, that were not detected in MBR permeate. More LRV data is required for protozoan removal by MBR. The combined set of all protozoan pathogens and indicators (21 detected / 26 total values) yielded a 5th percentile LRV of 3.5.

2.3 Properties of pathogens and indicators

Indicators are often chosen in place of pathogenic microorganisms due to their, relative abundance in source water (allowing smaller sample volumes for detection) and lower expertise requirement for assay. Indicator organisms can often be cultivated, allowing for challenge testing. *Clostridium perfringens* has been proposed as an indicator for protozoan removal due to its ability to form spores and resist bio-predation. There is some concern of the use of clostridia being too conservative, as it is 5 – 10 times smaller than the target pathogen, cryptosporidium. Size, abundance and IEP of pathogenic and indicator viruses were summarized in Table 9.

Indicator organisms, FRNA Bacteriophage (Includes MS2, Qβ) and somatic coliphage (includes ΦX174) are commonly used as surrogates for viruses (Marti *et al.* 2011). These are viruses that infect *E. coli*. Depending on the host *E. coli* chosen, different bacteriophages will be detected. FRNA bacteriophages can be differentiated from somatic coliphages by their method of replication. That is, somatic coliphages replicate by attacking the cell wall of *E. coli* hosts while FRNA bacteriophages attack the F.pili; a reproductive extension only present during logarithmic host growth phase (Grabow 2001). The unlikely event of replication of FRNA bacteriophages in water, combined with the fact they are excreted by humans, and are morphologically similar to smaller enteric viruses, has driven their use as indicator organisms.

Wild somatic coliphages can range in size from 27 – 100 nm (Wu *et al.* 2010). The *E. coli* host used for enumeration of somatic coliphages are *E. coli* C (ATCC:13706) or the naldixic acid resistant mutant CN13 derived from ATCC:13706 (Jofre 2009). The smaller somatic coliphage ΦX174 (27 nm) is a positive control for these hosts.

Somatic coliphage has been seen to exist at higher concentrations in the activated sludge when compared to the influent to an MBR (Marti *et al.* 2011) (Mosqueda-Jimenez *et al.* 2011). Previously, the higher MLSS density of somatic coliphage has been explained by the ability to infect a variety of *E. coli* hosts and replicate in the environment (Grabow 2001). The potential for replication has been disputed, as in most cases, there should not have been sufficient concentration somatic coliphage hosts to allow replication (Jofre 2009). Consequently, an explanation for whether accumulation of somatic coliphages within the activated sludge of MBR is due to, growth or resistance to biological predation, is still unavailable. Regardless of the reason for accumulation of somatic coliphage, the LRV demonstrated is of a conservative nature, due to the fact the membrane is being challenged at a higher concentration, than that of the feed water.

Table 9 was created to allow comparison of typical behavior and properties of common virus indicators and pathogens to facilitate selection of appropriate indicators.

Table 9 - Properties and abundance of indicator and pathogenic viruses.

Organism	Size (nm)[1]	Isoelectric Point[2]	Bioreactor concentration relative to Feed	Abundance in Sewage (Org/L)[3]
Virus Indicators				
Somatic Coliphage	ΦX174 27[4] 27 – 100 [5]	ΦX174: 6.6[4]	↑	$10^6 – 10^9$
FRNA Bacteriophage	MS2 24 [4] Qβ 24	MS2: 3.9 [4] Qβ: 5.3	↓	$10^5 – 10^7$
Pathogenic Viruses				
Adenovirus	70 – 90	Human Adenovirus C: 4.5	↑[6]	$10^1 – 10^4$
Norovirus G1	20 – 40	Norwalk virus: 5.5 - 6	≈ [7]	$10^1 – 10^4$
Norovirus G11	20 – 40	Norwalk virus: 5.5 - 6	↘[6] ≈ [7]	$10^1 – 10^4$
Rotavirus	60 – 80	Rotavirus A: 8.0		$10^2 – 10^5$
Enterovirus	18 – 27	Human Enterovirus C: Dual IEPs 4.8 and 6.1	↑[6]	$10^2 - 10^6$

2.4 Conclusions

Where possible, normal distribution parameters were fitted to data from the literature survey. These parameters may be used as a basis for decision of default LRV values for MBR. The 5th percentile LRV for the combined set of virus, bacteria and protozoan indicators were 1.7, 3.5 and 3.5 respectively. Guidance is requested from Sub Project 5, regarding the adequacy of the normal distribution parameters presented.

Of the two most common groups of virus indicators (i.e. somatic coliphages and FRNA bacteriophages), somatic coliphages exhibited the highest resistance to biodegration, effectively challenging the membrane at a higher concentration. It is uncertain if somatic coliphages are capable of growth within the activated sludge. Regardless, if a conservative indicator is sought for viruses somatic, coliphage may be a more appropriate choice than FRNA bacteriophages.

[1] Sizes taken from virus size document (P. Monis) emailed on 4/12/2013 by C. E. Robillot unless otherwise specified.
[2] (Michen and Graule 2010)
[3] Taken from AGWR 2006 Table 3.6
[4] (Dowd, Pillai et al. 1998)
[5] (Wu, Li et al. 2010)
[6] (Simmons, Kuo et al. 2011)
[7] (Sima, Schaeffer et al. 2011)

3 Review of Online Monitoring Options for MBR

3.1 Introduction

To effectively ensure recycled water quality, critical control points (CCP) must be identified and appropriate monitoring solutions implemented. The most common CCP for MBR has been membrane integrity, due to the significant role of the membrane in removing pathogens relative to the activated sludge. Common methods for assessment of membrane integrity are reviewed in the following sections.

3.2 Turbidity

Turbidity is a measure of 'cloudiness' of water. Turbidity via measurement of light scatter at 90°, is common in the water industry, is known as nephelometry. The nephelometric turbidity unit (NTU) scale was developed based on an agreed turbidity value of formazin standards (Gregory 1998). Conventional turbidimeters use white light from a tungsten lamp, while laser turbidity meters use specific wavelengths to enhance detection limits (Anon. 2012).

Due to its convenience, 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 1mNTU. It is noted that turbidity features lower cost, but also lower sensitivity when compare to particle counting (Guo *et al.* 2010). In a pilot scale experiment, a laser turbidity unit was capable of detecting 1 cut fibre out of 300, when challenged with latex microspheres and MS2 phage. The loss in integrity resulted in a drop in MS2 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). Mosqueda-Jimenez *et al*. compared log removal distributions for virus and bacterial indicators 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 faecal coliforms) were above 3.1 and 4.8 respectively (Mosqueda-Jimenez *et al.* 2011).

A cost effective solution to address the sensitivity limitation posed by dilution of contaminated flow through a defect was shown with a multiplexed turbidity system with one detector connected to multiple monitoring locations via fibre optic cable (Naismith 2005). Laser turbidity was reported capable of detecting 1 broken fibre 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 will exceed 12 NTU, potentially increasing sensitivity. Frequent calibration and maintenance checks are required to ensure accurate turbidity readings (Farahbakhsh *et al.* 2003).

A majority of online monitoring literature has focused on MF or UF membrane filtration, not necessarily on MBR; with optical techniques, such as turbidity, found less sensitive due to low feed water particle concentrations. While turbidity meters are not designed to directly measure pathogen concentration, they are expected to detect ingress of suspended solids. The activated sludge compartment of an MBR has a substantial concentration of particles. Literature has suggested that a majority of pathogenic viruses are adsorbed to MLSS (Section 1.2.2), suggesting that if failure of the membrane occurs, a decrease in LRV may be coincidentally shown by an increase in turbidity.

3.3 Particle counting

Particle counters monitor the concentration of particles within certain size ranges using laser based light scattering (Guo *et al.* 2010). Typical particle counters are optimised for detection of 1 to 100 particles > 2 µm/mL (Carr *et al.* 2003). Particle counting has been employed to monitor membrane plants; with membrane integrity demonstrated by comparison of feed and permeate 1 µm particle concentrations (Kruithof *et al.* 2001). Particle counting has been reported to indicate maximum log removals of 3.5 (Johnson 1998), up to 4.5 (Kruithof *et al.* 2001) in UF plants. Particle counter minimum detection levels, based on a 3 standard deviations from a plot of $\log_{10}$ [dosed particles/baseline] were reported for particle sizes 5, 1 and 0.025 µm respectively as 0.56, 2560 and 8.81×10^9 particle count/mL (Carr *et al.* 2003). From pilot trials, 50 m^2 of membrane area connected to an online particle counter was estimated as the maximum possible area where damage to one fibre could be detected (Adham *et al.* 1995). The main limitation on the sensitivity of particle counting was

attributed to low feed particle concentration; this is not likely to be a problem for MBR monitoring due to high MLSS. Another sensitivity issue with particle counting, as with turbidity, is the dilution of the contaminated flow through a compromised portion of membrane by the uncontaminated flow through intact membrane area. Similar interferences exist in particle counting as with turbidity namely, air bubbles in permeate or particles shed from permeate piping (Farahbakhsh *et al.* 2003).

Several steps have been noted as necessary to improve continued and accurate operation of particle counters (Guo *et al.* 2010):

- Connection tubing material should present minimal adsorption sites for target particles;
- Sensors must be cleaned and electronic background noise checked at regular intervals; and
- Flow control devices with strict tolerance should be installed between permeate pipe and particle counter to maintain required stable flow.

3.4 Pressure decay testing

Pressure decay testing (PDT), is available for hollow fibre membrane systems if, a means of providing air pressure in the permeate line is available. Permeate withdrawal must be stopped for a PDT. Hence, PDT can be classed as semi online. To achieve PDT in MBRs, the lumen side of hollow fibres must be pressurised to below the bubble point of the membrane (c. 200 kPa) (Johnson *et al.* 2003). Decline in pressure for a set amount of time is then observed; the overall test was reported to take 5 - 10 min (Johnson 1998); The rate of pressure loss following a logarithmic relationship with microorganism rejection (Judd 2011). Typical PDT frequency is 4 – 6 hr (Banerjee *et al.* 2001).

The resolution afforded by PDT was reported to achieve up to the equivalent of 5 log removal (Johnson 1998). Unfortunately, this was assuming contaminant particle size equivalent to protozoa, not viruses (Johnson *et al.* 2003). A pressure of 100kPa was estimated as the bubble point, corresponding a defect size of 0.8 – 4 µm, depending on membrane material (Oxtoby 2003). A defect of this size would permit infiltration of bacteria and viruses but would likely reject protozoa. An estimate of the initial pressure required for detecting a defect of 0.01 µm was given as 27.6 MPa, much larger than the mechanical strength of any existing low pressure membrane system (Walsh *et al.* 2005). Additionally, the sensitivity of PDT is governed by the sensitivity of installed pressure transducers and the ratio of pressure transducers to connected membrane area. Presence of a non-fully wetted membrane may induce a false positive for a PDT (Guo *et al.* 2010).

PDT may appropriately indicate defects > 2 µm in an MBR, however, an increased pressure decay rate does not always correlate to a reduction of LRV. The poor correlation between PDT and LRV was explained due to shielding of defects by MLSS, and retardation of pathogen passage (Mosqueda-Jimenez *et al.* 2011).

3.5 Other techniques

3.5.1 Membrane integrity sensor

The Membrane Integrity Sensor (MINT Sensor) was developed by MINT Pte Ltd Singapore. When employed in monitoring mode, the MINT sensor compares the resistance generated across a 0.45 µm PVDF membrane by monitoring Pressure 1 (Sample Feed Pressure) and Pressure 2 (Sample Permeate Pressure).

From Pressure 1 and Pressure 2 the 'C metric' is calculated and logged within the software internal to the MINT sensor using Equation 2.

Equation 2 - Calculation of the C metric for the MINT sensor

$$C\ metric = \frac{P1 - 2 \times P2}{P1}$$ Equation 2

The software within the MINT sensor then compares the rate of change of the C metric with previously collected rates of change. An increase in the rate of change of the C metric indicates the presence of foulants on the membrane internal to the MINT sensor. As the pore size of the MINT

sensor membrane is greater than that of the upstream membrane unit, this may indicate an integrity breach of the upstream membrane unit.

The MINT sensor can also be operated in 'LRV mode'. To operate in this mode serial dilutions of membrane feed in permeate must be challenged through the MINT sensor at set feed and outlet pressures. The time taken for each different dilution to reach the control limit of C = 0.7 is then used to generate a calibration curve. The failure time is then correlated with LRV (MINT_Pte_Ltd 2011). Two studies have been published regarding the development and application of the MINT Sensor (Krantz *et al.* 2011) (Phattaranawik *et al.* 2008). The MINT sensor was reported to be able to detect a break in one fibre out of 1500 fibres during a validation experiment at Bedok NeWater treatment plant Singapore (Krantz *et al.* 2011).

3.5.2 Spectroscopic techniques

Permeate colour and manganese, determined with spectroscopic methods, and dissolved organic carbon (DOC), analysed via the standard method, were reported to increase significantly during operation of a UF membrane with three pinhole defects (Walsh *et al.* 2005). Exploitation and correlation of UV spectra have made assessment of various bulk water quality parameters possible. These parameters include total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS) and nitrate (Vaillant *et al.* 2002). Studies have illustrated a correlation between UV absorption at 254 nm and TOC providing turbidity is low (Vanrolleghem *et al.* 2003). It is yet to be demonstrated whether commercially available online spectroscopic analysers can be used to provide a suitable control limit for membrane integrity monitoring via utilisation of these correlations. As a majority of DOC removal occurs within the activated sludge of an MBR (Lesjean *et al.* 2011), there may be capacity for spectroscopic techniques to act as a CCP for activated sludge health.

3.6 Conclusions

Online monitoring techniques exist for monitoring membrane integrity, for all configurations of MBR. What is unknown is the limit of detection and correlation of these techniques, with regards to pathogen breakthrough. Research is needed, specific to MBR, to assess the ability of these techniques at determining MBR log removal value. PDT is a well-established method of measuring membrane integrity. PDT is not applicable to all configurations of MBR and requires extreme test pressures to resolve virus sized membrane breaches.

No technique is commercially available for direct detection of pathogens. Due to the trend of viruses associating with suspended solids, there is a basis for use of optical techniques such as turbidity and particle counting in coincidentally indicating the breakthrough of pathogens. A key difference with the use of optical techniques in MBR is that a membrane breakage will allow a more substantial amount of suspended solids through, when compared to a direct UF system, due to the high concentration of suspended solids in the activated sludge.

4 Hazardous Events and Risk Management for MBR

4.1 Introduction

In conjunction with SP5, risk management practices with a focus on Hazardous events were reviewed in order to identify any potential conditions that could reduce performance of MBRs in water recycling. The complete findings of this section were published elsewhere (Trinh *et al.* 2014).

The operational performance of any wastewater treatment system can be viewed from two distinct perspectives. The first, and most commonly considered, is the inherently variable treatment performance that may be achieved when the system is operating within a defined set of 'normal' operational conditions. The less commonly considered perspective regards the consideration of how the system may perform in the event of a disruption to normal operating conditions. In the field of risk assessment, a departure from normal operational conditions is commonly termed a 'hazardous event'. Hazardous events that may affect the operation of wastewater treatment systems can include sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions.

Since hazardous events may occur from time to time, and may have significant impacts on short-term operational performance, the characterisation of the likelihoods and consequences of these events is necessary in order to fully characterise the long-term performance of the system. Indeed, hazardous event scenarios are commonly the scenarios that present the greatest levels of risk related to final water quality. Therefore, characterisation of these events is required to properly characterise risks including those posed to the environment and to human health.

The vast majority of observed waterborne disease outbreaks in developed countries during the last few decades have been associated with hazardous events, such as unusual weather patterns, plumbing errors or treatment failures (Hrudey *et al.* 2007, Rizak *et al.* 2007). Consequently, the assessment of hazardous event scenarios has become an integral component of drinking water quality management in many countries. This approach is encapsulated within the Australian Drinking Water Guidelines (NWQMS 2011) and the World Health Organization Guidelines for Drinking Water Quality (WHO 2011).

Following this trend in drinking water management, the Australian Guidelines for Water Recycling (NRMMC & EPHC 2006) have adopted a consistent approach for the qualitative incorporation of hazardous event analysis in overall system performance assessment. In this context, potential hazardous events are identified and each is allocated a qualitative measure for both its perceived 'likelihood' (Table 10) and its 'consequence' or impact (Table 11).

Table 10 - Qualitative measures of likelihood (NRMMC & EPHC 2006).

Level	Descriptor	Example description
A	Rare	May occur only in exceptional circumstances. May occur once in 100 years
B	Unlikely	Could occur within 20 years or in unusual circumstances
C	Possible	Might occur or should be expected to occur within a 5- to 10-year period
D	Likely	Will probably occur within a 1- to 5-year period
E	Almost certain	Is expected to occur with a probability of multiple occurrences within a year.

Table 11 - Qualitative measures of consequence or impact (NRMMC & EPHC 2006).

Level	Descriptor	Example description
1	Insignificant	Insignificant impact or not detectable
2	Minor	Health – Minor impact for small population Environment – Potentially harmful to local ecosystem with local impacts contained to site
3	Moderate	Health – Minor impact for large population Environment – Potentially harmful to regional ecosystem with local impacts primarily contained to on-site.
4	Major	Health – Major impact for small population Environment – Potentially lethal to local ecosystem; predominantly local, but potential for off-site impacts
5	Catastrophic	Health – Major impacts for large population Environment – Potentially lethal to regional ecosystem or threatened species; widespread on-site and off-site impacts

Once a suitable qualitative measure of likelihood and consequences has been allocated to each identified (potential) hazardous event, a qualitative risk estimation or 'risk rating' can be applied according to the risk matrix presented in Table 12 - Qualitative risk estimation (NRMMC & EPHC 2006).. The specific characterisation (e.g., low, moderate, high, very high) of risks relating to various combinations of likelihood and consequence measures may be adapted for particular systems and applications. The example given in Table 12 - Qualitative risk estimation (NRMMC & EPHC 2006) is that used in the Australian Guidelines for Water Recycling (NRMMC & EPHC 2006) and is very similar to those presented in the Australian Drinking Water Guidelines (NWQMS 2011) and the World Health Organization Guidelines for Drinking Water Quality (WHO 2011).

Table 12 - Qualitative risk estimation (NRMMC & EPHC 2006).

	Consequences				
Likelihood	1-Insignificant	2-Minor	3-Moderate	4-Major	5-Catastrophic
A Rare	Low	Low	Low	High	High
B Unlikely	Low	Low	Moderate	High	Very high
C Possible	Low	Moderate	High	Very high	Very high
D Likely	Low	Moderate	High	Very high	Very high
E Almost Certain	Low	Moderate	High	Very high	Very high

This risk assessment process provides a basis for managing risks and applying preventive measures. In the context of wastewater and recycled water management, preventative measures most commonly refer to actions, activities and processes used to prevent significant hazards from being present in final effluents or to reduce the hazards to acceptable levels. Risk should be assessed at two levels:

- Maximum (unmitigated) risk, which is risk in the absence of preventive measures — assessment of maximum risk is useful for identifying high-priority risks, determining where attention should be focused and preparing for emergencies.
- Residual risk, which is risk after consideration of existing and proposed preventive measures — assessment of residual risk provides an indication of the safety and sustainability of the system or the need for additional preventive measures.

The following sections are intended to provide insights to the potential impacts of hazardous events on the ongoing performance of membrane bioreactors. It is proposed that this information will be significant value to system managers, people responsible for system performance assessment and validation, health and environmental regulators and, ultimately, to the designers and manufacturers of future, more resilient systems.

4.2 Characterisation of potential hazardous events and their impact on MBR operation

In order to characterise hazardous events relevant to MBR operation, it is first necessary to describe the elements of an MBR process in relation to hazard analysis terminology. The primary hazard within the MBR process is presented by the components of the mixed liquor solution of an activated sludge system. In particular, pathogenic microorganisms within the activated sludge constitute a human health hazard, while bulk parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS) present environmental risks. The concentration of pathogenic microorganisms in activated sludge has been observed to be similar to sewage for indicator species prone to biological predation, such as *E. coli*. However, indicators that exhibit resistance to biological degradation and are of greater diameter than the membrane pore size, such as *Clostridium perfringens*, have been shown to accumulate within the activated sludge (Marti *et al.* 2011, van den Akker *et al.* 2012). As a result the concentration factor for resistant pathogens and indicators is expected to be proportional to the MBR solid retention time (SRT).

Due to the health and environmental hazard associated with the components of the mixed liquor, hazardous event scenarios are expected to include any deviation from normal MBR operation, which would lead directly, or indirectly to 'loss of containment' of the activated sludge. Loss of containment in MBR is expected to result from membrane/module integrity failure, overflow from the bio- or membrane reactor or decrease in the treatment efficiency of the activated sludge system. A range of threats could be defined within the various treatment steps of the MBR plant (Collection, Pre-treatment, Activated Sludge Process, Membrane and Post Treatment).

4.2.1 Collection

Collection of MBR influent may occur downstream of primary settling or pre-screening at a municipal wastewater treatment facility or following an equalisation tank in smaller decentralised systems. Nominal feed quality will be subject to diurnal, seasonal and regional variations. Shock loadings have been also widely reported to occur within the sewage collection, generally due to upstream intermittent discharge from industry, heavy rainfall event or via ingress into aged and damaged sewer mains.

Shock loads resulting from seawater ingress (Severn 2003), unregulated upstream discharge of industrial wastes and high loadings of non-dissolved material during storm weather flow were reported to affect nominal operation of MBRs (van Bentem *et al.* 2007). Maintenance cleaning of upstream unit operations, without appropriate isolation, can also result in shock loading of downstream processes with high concentrations of suspended solids and grease causing clogging of pre-treatment equipment and membrane units (Lazarove *et al.* 2008).

4.2.2 Pre-treatment

Arguably one of the most important aspects of operation of MBR, pre-treatment of sewage with fine screening (1 - 3 mm) with the possible addition of micro sieving (down to 250 µm), grit and grease removal is essential to preserve the integrity of downstream membranes. Bypass of screens due to seal and screen failure or even deliberate screen removal has been reported and can increase the likelihood of membrane damage by foreign materials (metal shavings, fibrous rag material, leaves, etc.).

Failure of fine screening caused accumulation of solids and grit in the membrane compartment leading to increased membrane cartridge damage and replacement rate; up to 50% of the inventory reported by Nishimori *et al.* (2010). Self-cleaning micro sieve systems can also pose a source of abrasive contaminants through loss of brush fibres during operation (van Bentem *et al.* 2010).

4.2.3 Activated sludge process

Threats to activated sludge include loss of aeration and circulation due to port clogging, mechanical fault or power loss and overdose of membrane cleaning chemicals (Judd 2011). Disturbances, particularly to influent quality, can result in foaming, leading to potential loss of containment via overflow of the aeration tanks. Simulations of hazardous events on activated sludge in MBR have revealed decreased capacity for removal of bulk parameters such as BOD, COD and total nitrogen,

however, simulations of microbial quality of the permeate was not possible with the model utilised (Friedler *et al.* 2008).

During operation, biological treatment processes may be exposed to changing environmental conditions such as variations in the flow rate, concentration, and quality of the raw wastewater entering the process. In general, any rapidly occurring or immediate change in the chemical or physical environment might be classified as a system 'shock'.

Organic shock loads have been described in terms of quantitative shock loads and qualitative shock loads (Gaudy *et al.* 1961). Quantitative shock load implies a rapid increase in organic loading by rising high concentration of substrate to which the sludge is acclimated or to which it needs no acclimation (Gaudy *et al.* 1961). However, waste streams do not often have constant chemical composition of the organic constituents. A qualitative change in the chemical composition of the substrate (with constant TOC concentration) may constitute a serious type of system shock. This is termed a qualitative shock load (Gaudy *et al.* 1961). It implies that the composition of the carbon source has changed from that to which the sludge is normally acclimated while it does not imply that the change is toxic. For example, the substrate may change from a predominantly carbohydrate waste to a proteaceous or a fatty waste, from simple sugars to polymers, or from sucrose to lactose.

An important variation on quantitative shock loads is 'starvation shock'. Most treatment systems are designed to manage some variability in flow regimes. However, in extreme conditions, some treatment plants exhibit feed starvation periods during which no appreciable wastewater feeds the systems. This discrepancy between the conceptual design and the practical situation may lead to process upsets and unsatisfactory system performance (Beler Baykal *et al.* 1990).

Toxic shock involves an influx of organics or inorganic constituents and radicals, which wholly or partially inhibit or damage the existing metabolic pathways or disrupt the established physiological condition of the microbial population (Gaudy *et al.* 1961). Rapid changes in pH of the waste are also considered to be in this class of shock loading although they are more easily controlled and may be of less significance than other toxicity shock loads.

Waste streams with high ammonia concentration are very commonly produced by human handling (Campos *et al.* 2002). Sudden increase in ammonia concentration in biological treatment process can be due to increase ammonia concentration in raw sewage or inhibition of nitrification in the biological treatment process (Hart *et al.* 2003). Similarly, pH changes in biological treatment processes can be due to pH variation in raw sewage or due to failure of denitrification process within the biological treatment units.

Temporary interruptions to aeration of MBR systems would be expected to have a detrimental impact on the aerobic metabolic degradation of chemical contaminants and potentially lead to change within the microbial community. Loss of aeration may also lead to loss of suspension of the MLSS, potentially causing damage to MBR membranes.

4.2.4 Membrane filtration

Crucial threats at the membrane filtration stage regarding the containment of activated sludge can be encompassed within the integrity failure of the membrane or the module itself (seals, gaskets, connections). Through fault tree analysis based on the top event of cryptosporidium release, threats were scoped for an ultrafiltration plant (Beauchamp *et al.* 2010) and can be equally applicable to the membrane filtration step of a MBR.

In addition to the case of membranes exhibiting manufacturing defects, solid particles and foreign bodies within the bioreactor can breach or damage the membrane. Moreover, inappropriate high dosing of cleaning chemicals, and pressure shock (due to air from integrity testing or water from hydraulic shock of a pump start up) are expected to increase the likelihood of membrane integrity failure (Beauchamp *et al.* 2010). Integrity failure can be induced via sparks from welding in the vicinity of membranes (Ayala *et al.* 2011) and high pressure hosing during maintenance cleans (Le-Clech *et al.* 2005).

Failure of the module integrity results in short circuit of the membrane by constituents present in the mixed liquor. Module weak points include seals couplings and membrane-frame/pot interface. Module

failure likelihood is increased as a result of the seal being of poor quality or inevitable wear out due to an insufficient replacement regime. Coupling failure of MBR cassette has been previously attributed to the strong mechanical forces in the module header due to the air-cycling fouling mitigation system (van Bentem *et al.* 2007).

The cleaning regime frequently imposed on membranes to remove fouling and recover hydraulic performance result in gradual changes in the physical and chemical membrane properties (especially decrease of mechanical strength) (Hajibabania *et al.* 2012). A decrease in mechanical strength of the hollow fibre membrane is expected to significantly increase the likelihood of membrane integrity failure.

4.2.5 Post treatment

MBR permeate is sometimes disinfected and/or stored shortly before discharge. The major post treatment threat can be defined as the bacterial regrowth in permeate lines or storage reservoirs, which have been reported to cause detectable levels of total coliforms in the permeate of MBRs (Zhang *et al.* 2007).

4.3 Expected consequences of key hazardous events types

Very little research has been reported to specifically examine the consequences of hazardous events to MBR performance. However, many insights can be obtained from previous studies of conventional activated sludge systems since the biological characteristics of the two types of systems are similar. The following sections discuss the expected impacts of hazardous events on the removal of chemical and microbial constituents, with observations derived from studies on both MBR and conventional activated sludge systems.

4.3.1 Impact on the removal of bulk organic matter and nutrients

Consequences of hazardous event conditions on conventional activated sludge and MBR treatment performance are summarised in Table 13. Results of quantitative organic shock load studies to activated sludge treatment systems show that reactors which were operated stable at influent COD concentrations above 100-500 mg/L can withstand influent shock concentrations of up to 1500 mg/L COD, even when the shock durations varied from hours to weeks (Gaudy *et al.* 1961, Saleh *et al.* 1978, Normand *et al.* 1981). However, at influent shock concentrations around 3000 mg/L COD, the change may exceed the maximum assimilation capacity of the biomass, leading to an increased deterioration of effluent quality caused by loss of biological solids (Saleh *et al.* 1978, Manickam *et al.* 1985). A 3000 mg/L COD shock load to an AS system was reported to cause a rapid growth in biomass, a noticeable change in colour of the mixed liquor, a decrease in floc size, an increase in filamentous forms and a reduction in the number of protozoa (Saleh *et al.* 1978). Disruption in COD removal capacity and the change in colour of an AS system were observed to be correlated with changes in the biochemical composition of the sludge (Manickam *et al.* 1985). In general, high organic concentration in influent wastewater is known to inhibit nitrification as it supports the growth of heterotrophic bacteria, which compete with autotrophic nitrifying bacteria for oxygen, nutrients and space.

Studies have shown that biomass concentrations have decreased sharply during the first four days of a starvation shock and then reduced more slowly after that (Urbain *et al.* 1993, Coello Oviedo *et al.* 2003). In addition, the bacteria cell size was also found to be reduced, which was described as one of the adaptive responses to starvation conditions (Kjelleberg *et al.* 1987, Urbain *et al.* 1993, Coello Oviedo *et al.* 2003). These responses were related to the degradation of both proteins and polysaccharides contents of the sludge and led to a decrease in respiratory activity of the microorganisms. After 3-4 days under starvation conditions, the biomass drastically lost its ability to biodegrade exogenous nutrients reactions (Urbain *et al.* 1993). Starvation shocks also resulted in disappearance of some of the typical microbial groups usually found in an activated sludge, and appearance of other opportunistic microorganisms (Coello Oviedo *et al.* 2003).

The removal efficiencies of COD, TOC, total suspended solid (TSS), total Kjeldahl nitrogen (TKN) and phosphate by a MBR were reduced significantly under a feed starvation shock load of 5 days (Yogalakshmi *et al.* 2007). In addition, a large fraction of biomass wash off and a reduction in microbial activity inside the reactor was observed. The removal of organics and nutrients was recovered back to steady state conditions after 6 days of normal operation. However, it took nearly a

month of continuous operation to regain the amount of biomass lost during feed starvation shock load (Yogalakshmi *et al.* 2007). High salt concentrations in a biological reactor have been reported to reduce organic removal efficiencies and biomass settleability (Dan *et al.* 2003, Ng *et al.* 2005). This is because salty conditions produce high osmotic pressure on bacteria cells, which can inhibit bacterial growth and floc formation (Dan *et al.* 2003). Additionally, high salt concentration conditions also reduce gravity separation due to lower density difference between water and biomass (Ng *et al.* 2005).

Failure modes leading to physical membrane damage tend to be gradual rather than sudden and are easily identified by long-term changes in flux or operating pressures. Accordingly, their relevance as 'hazardous events' leading to sudden deterioration in water quality appears low. Nonetheless, there is some evidence to suggest that events such as chemical membrane cleaning and accidental exposure to excessive chlorine concentrations may physically harm some types of water treatment membranes leading to reduced performance (Simon *et al.* 2009, Beyer *et al.* 2010).

4.3.2 Impact on the removal of microorganisms and microbial indicators

Information on the impact of hazardous events on the removal of pathogenic microorganisms by MBRs is scarce. Research has traditionally focused on studying the behaviour of microbial indicators (model organism) under a range of event conditions. Most of this information has been derived from lab- and pilot-scale studies, whereby key operating parameters can be easily adjusted and challenged under controlled conditions. The impact of key operational events on the microbial removal efficiency of MBRs are summarised in Table 14. What is clear from Table 14 is that the most important mechanisms responsible for removing microorganisms are membrane rejection and biodegradation. Generally, pathogen removal improves as membrane fouling layers develop, and thus events that lead to the removal or disturbance of fouling layers (e.g., membrane cleaning, backwashing and change in permeate flux) can adversely influence removal. The extent of membrane fouling is commonly quantified by the monitoring changes in the permeate flux or the transmembrane pressure (TMP).

MBRs are well known for their ability to remove a wide range of model indicator organisms (e.g., bacteria, phage and spores) and what is clear from the literature is that each organism behaves differently. Notably, the removal of membrane fouling influences the rejection of phage more so than bacteria; simply because phage are much smaller than the pore size of membranes. As a result, phage removal is typically less consistent and is more subject to the type of membrane and its pore size (microfiltration vs. ultrafiltration) and to changes in operation, such as membrane TMP, permeate flux and spikes in initial feed concentrations. Therefore, bacteriophage appear to be a superior model organism for understanding the impacts of hazardous event conditions on the microbial removal efficiency of MBRs.

Not all phage species behave the same. Different species feature varying retention mechanisms, owing to differences in surface properties. For example, F-specific phage have a higher tendency to adsorb to membrane surfaces and suspended biomass more so than somatic phage, exhibiting a more even removal pattern during maintenance cleaning events (Zhang and Farahbakhsh 2007). The removal patterns of native and laboratory-grown phage strains can also differ (Hirani *et al*. 2010). Selection of the right model organisms (i.e. one that shares a similar fate to target pathogen) is therefore crucial when characterising the impacts of hazardous events on MBR performance.

Research characterising the removal of model organisms by MBRs also suggests that the suspended biomass (mixed liquor) can play a very important role in the elimination of pathogens via adsorption and predation. The contribution of biomass, however, is dependent on inter-related parameters including the concentration of mixed liquor suspended solids, the sludge retention time and the food to mass ratio; and thus operational events that lead to changes in these parameters may influence pathogen removal. At this time, the relative impact of the fouling layer on the rejection capability of the membrane has still not been clearly demonstrated. The role of the irrecoverable fouling layer formed over years of continuous operation is expected to be responsible for the build-up of a protective layer suitable for adsorption for viruses. However, Table 14 indicates that the various types of cleaning used in MBR maintenance generally result in lower pathogen rejection.

Table 13 - Consequence of hazardous event conditions on AS and MBR treatment performance based on select studies.

Event Type	System	Monitored parameters	Consequence on removal	Ref
Organic shock	AS operated stably at influent COD of 100-500 mg/L	COD	Influent COD increased to ≤ 1500 mg/L: no impact	1,2,3
			Influent COD increased to ≥ 3000 mg/L: biomass grown rapidly, floc size decreased, filametous forms increased and number of protozoa reduced, loss of biomass causing deterioration of effluent quality.	2,4
Starvation shock	AS system subjected to 10 d starvation period	Biomass characteristics	After shock 8 d, biomass concentration and respiration activity decreased sharply due to degradation of proteins polysaccharides contents in biomass.	5
	AS system subjected to 21 d starvation period	pH, SS, VSS, CODd, DOC, biomass characteristics	Biomass concentration, bacteria cell size and respiration activity decreased sharply during first 4 d, disappearance of some typical microbial groups in AS. CODd and DOC in liquid phase increased sharply between day 4 and 9 due to release of organic material from death microorganisms.	6
	MBR system (hollow fibre, 0.4 µm) subjected to 5 d starvation period	COD, TOC, TSS, TKN, phosphate, biomass characteristics	After 5 d starvation, removal efficiencies of COD, TOC, TSS, TKN, phosphate reduced significantly and they recovered fully after 6 days of normal operation. Biomass concentration and activity reduced significantly and took a month to recover.	7
Salinity shock	AS system subjected to NaCl up to 45 g /L	COD, biomass characteristics	COD removal and biomass settleability reduced.	8
	As systems subjected to NaCl from 0 to 60 g/L	COD, biomass characteristics	NaCl ≤ 10 g/L: DOC removal slightly increased NaCl > 10 g/L: DOC removal reduced NaCL ≥ 15 g/L: morphological changes in microbial population NaCl ≥ 30 g/L: effluent turbidity increased	9

1.(Gaudy *et al.* 1961); 2. (Saleh *et al.* 1978); 3.(Normand *et al.* 1981); 4.(Manickam *et al.* 1985); 5. (Urbain *et al.* 1993); 6. (Coello Oviedo *et al.* 2003); 7. (Yogalakshmi *et al.* 2007); 8.(Dan *et al.* 2003); 9. (Ng *et al.* 2005)

Table 14 - Consequence of operational and event conditions on the removal of microbial indicators based on select studies.

Event Type	Membrane	Model Organisms	Consequence on LRV	Ref
Chemical backwash	Zenon ZW-500C-SMC	Somatic coliphage	Small decrease (from 3.0 to 2.5)	10
		F-specific coliphage	No significant impact	
Formation of fouling	Memcor 0.2 µm	Indig. somatic coliphage	Increase from 1.2 (clean) to 2.0 (fouled)	11*
			No impact	
Increase in flux for clean membrane			Decrease from 2.2 (50 Lm-2h-1) to 1.7 (85 Lm-2h-1)	
Increase in flux for fouled membrane			2.3 (25 Lm-2h-1), 2.7 (50 Lm-2h-1) and 2.3 (85 Lm-2h-1)	
Longer filtration/relaxation cycle	Six MBR systems	Seeded MS-2 phage	Increase from 2.9 (1 min cycles) to 3.4 (8-18 min cycles)	12
Relaxation period/air scouring			Small decrease by 0.25 LRV	
Change in pore size (0.03 – 0.1 µm)			Increase from 1.5 (0.1 µm) to 4.5 (0.03 µm)	
		Indig. coliphage	No impact, due to particle association	
Change in pore size (0.03 – 0.2 µm)	Nine MBR systems	Coliform bacteria	No impact	13
		Indig. coliphage	No impact	
Increase in MLSS conc. (3 to 9 g/L)	Hollow fibre, 0.4 µm	Indig. somatic coliphage	No impact on LRV, but change in biological action	14
Formation of fouling			Increase from 0.6 (clean) to 1.5 (fouled)	
Chemical backwash			Decrease by 0.5 (attributable to biomass only)	
Change in SRT (10 to 50 d)			Weak increase by 0.05 (attributable to biomass only)	
Change in HRT (8 to 13 hr)			Increase from 1.5 to 1.9 (attributable to biomass only)	
Filtration of supernatant	Flat sheet, 0.4 µm	T-even-like indig. phage	LRV across membrane only: 0.5	15
Operation with mixed liquor			Increase to 4	
Power failure to air scour and influent pump			Increase from 0.4 to 1.0, possibly due to increased fouling	
Clean membrane (filtration of supernatant)	Hollow fibre, 0.4 µm	MS-2 phage	0.3 – 0.4	16
High flux operation			Decrease	
Operation with mixed liquor			Increase to 1.0 (after 9 hr) to 2.0 (21 d)	
Change in MLSS conc. (6 to 10 g/L)			No impact	
Formation of fouling	Flat sheet, 0.4 µm	Indig. somatic coliphage	No significant impact	17
		Indig. FRNA phage	Increase from 4.5 to 4.8	
		Bacterial indicators (spores, *E. coli*)	No impact	

Event Type	Membrane	Model Organisms	Consequence on LRV	Ref
Membrane rinsing	Hollow fibre, 0.22 µm	T4 coliphage	Decrease from 5.8 to 3.1	18
Chemical cleaning			Decrease from 5.8 to 1.7	
Chemical cleaning	Hollow fibre, 0.22 and 0.1 µm	Coliphage f2	Decrease from 3.9 to 0.8	19

10 Zhang *et al.* (2007); 11. Farahbakhsh *et al.* (2004); 12.Hirani *et al.* (2010), 13. Hirani *et al.* (2012); 14. Wu *et al.* (2010); 15. Ueda *et al.* (2000), 16. Shang *et al.* (2005); 17 Marti *et al.* (2011); 18. Lv *et al.* (2006); 19. Zheng *et al.* (2006).
*Direct filtration of sewage, no MBR.

4.4 Assessing likelihoods of MBR hazardous events

Techniques for quantitatively assessing the likelihoods of specific hazardous events could be investigated including the use of historical data such as weather patterns and frequencies of power failures or mechanical malfunctions. An alternative approach is by the use of available mechanical reliability measures such as critical component analysis methodology (Shultz *et al.* 1982, Olivieri *et al.* 1996, Eisenberg *et al.* 1998, Eisenberg *et al.* 2001).

A critical component analysis can be carried out by creating a list of all components in a facility and then categorising the components by treatment unit, component and subcomponent. Data are collected for all planned and unplanned maintenance events and then used to compute performance statistics for treatment units and for individual components in the treatment system. The performance statistics describe the expected time between failures for treatment units, the overall mean time between failures of components, and the fraction of time that a unit or component was operating, either including or excluding preventative maintenance.

This type of analysis provides a foundation from which an assessment of the inherent reliability of a treatment system may be made. For example, if it can be demonstrated that a treatment facility is operational nearly 100 per cent of the time on a long-term basis, plant performance data may be used to evaluate the probability that the effluent will meet a specified set of criteria. Otherwise, it may be necessary to investigate if and how component failures impact treatment plant effluent quality.

The established engineering parameters Mean Time Between Failures (MTBF, a function of reliability) and Mean Time to Repair (MTTR, a function of availability) may be used to calculate the operational availability (Ao, the probability that an item is in an operable state at any time) as shown in Equation 3.

Equation 3: Determination of operational availability from MTBF and MTTR

$$A_o = \frac{MTBF}{MTBF + MTTR}$$ Equation 3

Reliability of machinery can be derived through parametric models to serve as population models for failure times arising from a wide range of products and failure mechanisms. Weibull statistics provide a life distribution model, which has been useful in many engineering applications to derive failure rates (Carrasco *et al.* 2008, Davis *et al.* 2008, Erumban 2008). The two-parameter Weibull distribution function has been used to derive a reliability function R(t) given by the cumulative form (Equation 4).

Equation 4: Reliability function R(t) from the cumulative form of the Weibull distribution

$$R(t) = \int_t^{\infty} f(x)dx = e^{-(x/\beta)^{\alpha}} \qquad t \geq 0,\ \alpha > 0,\ \beta > 0$$ Equation 4

Where α is the Weibull shape parameter, β is the scale parameter, and t is the time of operation.

The scale parameter β has the same units as t and the shape parameter α is a dimensionless quantity. When α=1, representing a constant failure rate, the reliability model is simplified to the form presented in Equation 5.

Equation 5: Reliability function R(t) for a constant failure rate (α=1)

$$R(t) = e^{-\lambda.t}$$ with the failure rate (λ), $$\lambda(t) = \frac{1}{\beta} = \frac{1}{MTBF}$$ Equation 5

Process reliability for an MBR system may be engineered through reliability assessments made using Weibull distribution databases for all mechanical components (Moore *et al.*

2008). Historical MTTR for each component can be tracked and updated through corrective maintenance work orders. The MTBF and MTTR values analysed may also form part of an asset replacement strategy.

4.5 Management of hazardous events through engineered redundancy and multiple barrier treatment systems

It is generally not possible to guarantee the prevention of many types of hazardous events. Accordingly, systems must be designed with a degree of robustness to manage impacts to ongoing operation as well as risks to human health and the environment when hazardous events occur. Important concepts for managing hazardous events are the incorporation of multiple barriers in the design and the establishment of a monitoring program that is suitable to constantly assess proper system performance. The selection of multiple barriers and a monitoring program will depend on the context in which an MBR is employed. Meeting effluent discharge standards will require a different management approach to potential hazardous events as compared to practices where MBR effluents are used for non-potable or potable reuse applications given the higher degree of potential exposure to public health.

Multiple barriers in water treatment and reclamation are aimed at ensuring that performance goals are met by (1) expanding the variety of contaminants a process train can effectively address by providing engineered redundancy (i.e., robustness) and (2) by improving the extent of consistent performance of a unit process to attenuate a contaminant (i.e., reliability) (National Research Council 2012).

Even when true redundancy is not provided, multiple barriers can reduce the consequences of hazardous events when they do occur. The independence of multiple barriers is a key aspect of system reliability and safety (Drewes *et al.* 2011). For example, to mitigate the risk from pathogen exposure, all MBRs usually employ a disinfection step either using a chlorine-based disinfectant or UV irradiation, in addition to the MF or UF membrane that serves as a barrier to pathogens.

The extent of system performance and water quality monitoring will depend on project-specific water quality objectives and the potential impact from hazardous events. An idealized monitoring program would measure critical process parameters and microbial and chemical contaminants in real time in the finished product water. However, real-time monitoring comes at significant capital and maintenance expenses and needs to be balanced against the estimated likelihood of certain hazardous events.

Monitoring requirements usually become more stringent (e.g., more frequent and broader in scope) as the potential for human contact with the reclaimed water increases (e.g., non-restricted irrigation of public parks; indirect potable reuse). Monitoring programs to assure that water quality requirements are met most commonly include effluent turbidity and residual chlorine. Operational parameters that are measured in real-time include flow measurements, transmembrane pressure, bioreactor tank levels, dissolved oxygen concentration of the bioreactor, as well as status of pumps and critical valves (i.e., on/off). These parameters are recorded in the Supervisory Control and Data Acquisition System (SCADA) of the treatment facility and usually linked to certain threshold levels. An exceedance of these threshold levels that might be caused by a hazardous event will result in shut-down of the system to mitigate the negative impact of that event.

4.6 Conclusions

The possibility or frequency of hazardous events plays a significant role in defining the overall risks to health and the environment from wastewater treatment by MBRs. Potential hazardous events are diverse and even prediction of a comprehensive suite of events that may disrupt and MBR performance is difficult. However, important examples include rapid and/or significant changes in influent water quality impacting the biological integrity and physical damage, which may impact membrane integrity. Hazardous events may lead to drastic loss of

treatment performance by impeding microbial degradation processes or by impeding the retention of particulate substances by membranes.

Formalised risk assessment procedures, aimed at rating potential hazardous events in terms of their likelihood and consequences are well suited for assessing MBR system vulnerabilities. Existing risk management approaches including the multiple barrier approach and a focus on monitoring the performance of operational parameters can be effective means for managing these vulnerabilities for the protection of health and the environment.

As important as the proper assessment and management of system failures and risks may be, surprisingly little attention has been paid to this topic for MBRs. More comprehensive future risk management will benefit from focused investigation of a wider range of potential failure modes, their consequences particularly in terms of their impacts to final water quality, and statistical descriptions of their likelihood. These factors will enable informed assessment of risks and better direct efforts towards more effective risk management.

5 Current state of validation practices in Australia

5.1 Introduction

In 2013, the Victorian Department of Health released the validation guidelines for pathogen reduction (VDoH 2013). In this study, these guidelines will be proposed as a starting point for the development of national validation guidelines. The following section contains key elements from the Victorian validation guidelines relevant to MBR systems. In order to better a better insight in the current validation process in other states, validation reports and recycled water quality management plans provided by MBR operators were also reviewed. Best practice elements from VDoH guidelines and supplied reports were summarised and contrasted to identify areas requiring consistency to facilitate the development of accepted national guidelines.

5.2 Key elements from Victorian validation guidelines

5.2.1 Approaches to validation

Two validation approaches are suggested for MBR:

1. Validating the system based solely on size exclusion by the membrane.
2. Validation of the MBR process.

Approach number 1 requires that the membrane can undergo direct pressure based integrity tests eg. PDT and that the validation is performed in clean water according to the USEPAs membrane filtration guidance manual (USEPA 2005) and section 6 of the VDoH. This approach cannot be considered for MBR configurations that are flat sheet, and present serious integrity concerns when applied to some hollow fibre systems.

Approach number 2 is applicable to all configurations of MBR and accounts for the additional removal mechanisms by the activated sludge. The requirements for approach number 2, will therefore be considered a priority and were reviewed in the following sections.

5.2.2 Microorganisms or surrogates for validation monitoring of MBR

Section 3.6.3 of VDoH details the nature of surrogates suitable for validation testing. Surrogates may be used in place of infection pathogens as:

- They can be easier to cultivate and use in seeding studies,
- Cheaper or quicker to assay, and
- Safer to handle.

A surrogate should be a challenge organism, particulate or chemical that is a substitute for the target microorganism of interest. A suitable surrogate should be removed by the treatment process to an equivalent or lesser extent than the target pathogen. If this cannot be achieved, it must be possible to demonstrate a reproducible correlation, from scientific literature, laboratory or field trials between the reduction of the surrogate and the target pathogen (over the LRV range required). Table 15 contains microorganisms of interest for validation of MBR reproduced from the Victorian validation guidelines.

Table 15 – Challenge test organisms listed in VDoH 2013.

Pathogen Group	Target Microorganism	Microbial Indicators
Viruses	Enteroviruses	Indigenous or seeded cultivable enteroviruses or indigenous or seeded coliphage, such as somatic or FRNA coliphage, may be used if demonstrated to be a suitable surrogate for in situ conditions (as per section 3.6.3). This relationship may be demonstrated at pilot scale.
Protozoan parasites	Cryptosporidium	Indigenous or seeded Cryptosporidium or Indigenous or seeded *Clostridium perfringens* may be used if demonstrated to be a suitable surrogate for in situ conditions (as per section 3.6.3). This relationship may be demonstrated at the pilot scale.
Bacteria	*E. coli*	indigenous or seeded *E. coli*

5.2.3 Verification and sampling requirements

The indicators from Table 15 should be taken as paired grab samples of influent and permeate samples to the MBR in triplicate for the operation conditions listed in Table 16.

Table 16 – Sampling requirement for MBR during validation testing VDoH 2013.

Period	Sampling Event	Filter cycle		
		Low Fouling (after chemical clean)	Medium Fouling	High Fouling (before backwash)
Over extreme seasonal periods (winter and summer) or intensive monitoring for worst case seasonal/diurnal period (if known based on evidence).	Number of paired samples per filter cycle	3	3	3
	Number of filter cycles (non-consecutive days)	6	6	6

The following physiochemical parameters are recommended for concurrent monitoring during the validation period: F/M Ratio, SRT, HRT, MLSS, pH, ammonia, DO, Temperature, permeate SS, permeate turbidity, Flux, TMP, and cross flow velocity.

Additional to the above samples, determination of a concentration factor of pathogens in the activated sludge; the concentration factor is level of challenge organisms in the mixed liquor relative to the feed water. Determination of this factor necessitates sampling of the activated sludge, no sampling frequency is specified. It is also necessary to take sufficient samples to demonstrate a correlation between online monitoring technique response and LRV.

5.2.4 Correlation of online monitoring technique with pathogen reduction

Where using indirect membrane integrity monitoring, such as turbidity, as a CCP for the MBR, it is necessary to correlate the LRV with the CCL. The approach suggested in the Victorian Validation guidelines is summarized below:

- Establish a correlation between permeate suspended solids, turbidity and surrogate microorganism concentration. A valid relationship will display a regression coefficient of > 0.9. This will identify the lower limit of online monitoring technique sensitivity.

- Sample pathogen concentration, suspended solids and permeate turbidity while progressively damaging a membrane.
- Calculate the permeate microorganism concentration where significant response is seen (above the base line from step one) and determine the LRV by comparison with the influent pathogen concentration.

5.2.5 LRV assignment following validation

LRV assigned to the MBR is the lowest value of either:

- LRV demonstrated during challenge testing calculated based on lowest LRV of paired triplicate samples or
- The maximum LRV that can be reliably verified by the integrity test (according to the correlation above)

5.2.6 Operational monitoring post validation

Ongoing operational monitoring should include:

- CCP determined from validation testing,
- Bulk parameters concurrently studied from validation period, and
- Weekly monitoring of permeate bacteriophage concentrations.

Post maintenance and prior to production of recycled water the MBR must be re-stabilised. Re-stabilisation must be verified by monitoring bacteriophage concentrations and physiochemical parameters.

5.2.7 Revalidation conditions

Generic (section 3.1) and MBR specific (section 7.3) conditions requiring revalidation are provided in of the VDoH including:

- Generic:
  - Design modifications,
  - Changes to control philosophy or operational parameters that deviate from the conditions of the prior validation.
- MBR specific:
  - Membrane replacement (New membranes should be subjected to the sampling program in Table 16 for at least 3 filtration cycles).

5.3 Key lessons from existing validation reports

A number of validation reports and recycled water quality management plans (RWQMP) have been obtained from our industry partners and reviewed here, with the key lessons learned collated here. The approach to validation, data provided and resulting LRV accreditation was summarised in Table 17.

Table 17 - Validation report documentation, approach and LRV credit.

Site	Documentation Provided to SP1	Validation Approach	LRV Credit
Site 1	Challenge test result	Challenge testing	Unknown
Site 2	Challenge test result Validation report RWQMP	Pre validation report Challenge testing	Virus 2 Bacteria 4 Protoza 4
Site 3	Pre-validation report	Pre-validation report Challenge testing T.B.A [h]	Virus 2 Bacteria 4 Protoza 4 Limited [h]
Site 4	RWQMP Supplier data	Pre-validation report Supplier data Previous validation of CAS pre upgrade	Virus 1.5 Bacteria 3.0 Protoza 2.0
Site 5	RWQMP Verification monitoring data	For discharge Not validated	None

Site 4 was validated based on historical performance of a parallel conventional activated sludge system, with supply of additional challenge test data from literature, concerning the membranes used. The LRV accreditation received by site 3 was 1.5 (Virus), 2.0 (Protozoa) and 3.0 (Bacteria). Site 5 provided verification monitoring of permeate and did not appear to intend on recycling produced water.

From pre-validation reports for Sites 2 and 3, LRV for virus was initially claimed at 2.5 (Membrane filtration default value Table 3.4 (NRMMC/EPHC 2006)).

Site 2 was accredited with an LRV of 2 (virus), 4 (Protozoa) and 4 (Bacteria) post challenge and verification of LRV (14 Week). Site 3 received the same accreditation for LRV without challenge testing. However, conditions were imposed including, limitations on maximum flux, and requirement to discharge permeate for 1 week following mains cleans. Site 3 is presently in the process of a challenge and verification step to address the operating limitations. Elements of reports provided were collated and provided below.

5.3.1 Pre-validation risk assessment

Pre validation risk assessments followed the following typical format:

- List of intended uses and users
- List of relevant licenses and legislation
- Scheme log reduction requirements according to end uses (commonly using Table 3.8 AGWR (NRMMC/EPHC 2006))
- Description of unit operations
    - Including operating envelope
- Description and justification for LRVs attributed to each unit operation
    - Taken from Table 3.4 of AGWR and/or
    - Scientific literature supplied and/or
    - Previous validation reports for similar systems
- Description of CCP and CCL
- Description of monitoring parameters

5.3.2 Validation challenge/verification testing

In cases where data presented in the pre-validation risk assessment has been found to not cover the operational envelope or, the intended uses of the scheme are 'high risk', challenge testing has been conducted.

[h] Accreditation at site 3 was limited to maximum operating flux of 25 LMH. Challenge testing to be conducted to confirm LRV at peak design flux of 32 LMH.

Challenge test data has covered the following elements:

- Identification of target pathogens and suitable surrogates
  - Commonly used surrogates have included *Clostridium perfringens* (protozoa), *E. coli* (bacteria) and somatic coliphage (virus)
- Identification of operating envelope and CCP parameters for parallel monitoring
- Identification of a sampling duration and frequency
  - Site 1 conducted challenge tests 3 times per week for 10 weeks
  - Site 2 conducted challenge tests once per week for 14 weeks including one wet weather event
  - Site 3 planned to conduct 22 sampling events over 5 weeks, 3 times a week for 4 weeks before membrane cleaning and 5 times per week for 2 weeks after membrane cleaning.

5.3.3 Recycled water quality management plan

RWQMPs have included the same elements as pre validation risk assessments with the addition of:

- Finalised log reduction targets
- Defined CCPs, CCLs and corrective actions for breach of a CCL
- Requirements for revalidation (Site 2 RWQMP)
  - Significant changes to influent quality
  - Changes to the treatment process
  - Changes to the intended use
  - Changes in legislation

5.3.4 Critical control points and monitoring criteria

Where available, the CCP and monitoring criteria were summarized below from the validation reports supplied.

Table 18 - CCP for MBR from validation reports.

Site	Parameter	Limit	Corrective Action
Site 1	Turbidity	< 0.2 NTU	Bypass to head of works until turbidity < 0.17 NTU
Site 2	Turbidity	>0.5 NTU	Bypass to farmers storage
Site 3	Turbidity Flux TMP Temperature	< 0.2 NTU < 32.5 LMH < 0.85 bar >13°C	
Site 4	Turbidity	> 0.2 NTU average for 24hours (warning) or >0.5 NTU for > 60 min	Membrane train shutdown and investigation

Table 19 - Post validation verification monitoring.

Site	Parameter	Frequency
Site 1	*E. coli* (post disinfection)	Weekly
Site 2	*E. coli* Faecal Coliforms *Clostridium perfringens* Somatic Coliphage	Weekly Weekly Weekly Monthly
Site 4	*E. coli* (post membrane)	Weekly
Typical parameters from all available site data	BOD, COD, SS, Colour, TDS, TN, NO_x, NH_3, Total Phosphorus, Reactive Phosphorous, Chloride, Total Residual Chlorine, Free Chlorine, SAR, Yearly Trace Organics (Cations, Anions)	Monthly

5.4 Conclusions

From review of validation reports and the VDoH, differences in the approach to validation across states and sites have been identified:

- The need of a challenge test period
- The validation sampling period and frequency
- Accredited log removal value assignment
- Operational monitoring post validation, for validation reports surveyed no reports assess permeate bacteriophage concentrations.

Where challenge testing has been conducted, the results are consistent with the microbial indicator surrogates listed in the VDoH, but the target pathogens have not been assessed. The proposed virus indicator, adenovirus for National MBR validation, is not listed in the VDoH.

6 Data collection from full scale MBRs

6.1 Introduction

A major limitation with existing LRV data from MBRs was the lack of accompanying operational parameters. As a result, identification of influencing factors on MBR LRV was not possible from literature alone. Accordingly, SP1 conducted a significant number of site visits at 9 full scale MBRs with a focus on collection and paring of operating parameters with LRV.

6.2 Site Visits and Sampling

A summary of the sites, number of sampling visits and usefulness of particular MBRs with respect to guideline development was included in Table 20.

Table 20 - Summary of site selection and number of visits conducted by SP1.

Sites	**# of sampling visits (total by end June)**	**Specific comments (in respect to use in guidelines)**
Ballina	17	• Flat sheet, large pore size, high flux, 4 independent permeate trains
Blacktown	9	• Flat sheet, gravity driven, 7 year old • No further visits due to difficulty obtaining monitoring data
Central Park	30	• Hollow fibre, high flux, intermediate SRT & HRT, < 1 year old, 2 independent permeate trains • Sampling for Cryptosporidium and Viruses • Online monitoring trial
Christies Beach	17	• Hollow fibre, high flux, intermediate SRT and HRT, 6 independent permeate trains, 2 years old • Chemical Cleaning study
Kangaroo Valley	3	• Hollow fibre • No further visits due to difficulty obtaining monitoring data
Kurrajong	3	• Flat sheet, gravity driven, < 1 year old • No further visits due to difficulty obtaining monitoring data
North Head	61	• High/constant flux, short HRT, short SRT, hollow fibre • Sampling for Cryptosporidium and Viruses • 10 year old membranes sampled before and after replacement • currently running with new generation
Pitt Town	40	• 3 year old, hollow fibre, long HRT, long SRT, high MLSS, low flux • Online monitoring trial • Chemical cleaning trian
Robertson	1	• Hollow fibre • No further visits due to difficulty obtaining monitoring data
Total Visits		**181**

At each site indicator microorganisms, bulk parameters and operational data as collected and analysed. Table 21 contains a summary of parameters and analysis undertaken for each site visit.

Table 21 - Sampling and data from each site visit.

Parameter Type	**Test**	**Sample Location**
Virus Indicators	FRNA Bacteriophage	Influent, Activated Sludge and Permeate
	Somatic Coliphage	
Bacteria Indicators	*E. coli*	
	Total Coliforms	
Protozoa Indicator	*Clostridium perfringens*	
Bulk Parameters	Dissolved Organic Carbon	
	Fluorescence Excitation Emission Matrix Spectra	
	pH	
	MLSS/VSS	Activated Sludge
	Capillary Suction Time	
	Dissolved Oxygen	*SCADA
	Turbidity	Permeate
Operating Data	Trans-membrane Pressure	SCADA
	Flux	SCADA
	SRT	SCADA
	HRT	SCADA

7 Techniques to identify influencing factors on MBR LRV

7.1 Introduction

Influencing factors on MBR LRV must be identified in order to recommend an appropriate validation methodology. Through collaboration with SP5, Bayesian analysis has been utilised to assess the large body of data resulting from the SP1 sampling program. As the use of Bayesian belief networks for the purpose of identifying influencing factors is relatively new additional approaches will be trialled. Presently, Artificial Neural Networks as well as simple correlation of operating parameters vs LRV is underway in order to assess agreement with the Bayesian approach. Final results will be available at a later date. The remainder of this section concerns the initial application of Bayesian network development and modelling to identification of influencing factors in MBR.

7.2 Constructing the MBR Bayesian Belief Network

7.2.1 Bayesian Belief Networks

Conventional data analysis methods such as plotting scatterplot and determining correlation coefficient like r^2 or Pearson r (ρ) can capture the relationship between two environmental parameters when a strong linear correlation exists, e.g. the scatterplot and ρ in Figure 4 demonstrate a clear linear relationship between mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) from 9 full-scale MBRs across Australia. However, scatterplot and ρ fail to clearly capture the relationship between multiple operational parameters such as dissolved oxygen (DO), hydraulic retention time (HRT), solid retention time (SRT), temperature, MLSS, MLVSS, and MBR permeate, as MBR treatment is a complicated dynamic process which is simultaneously affected by multiple factors including microbial, chemical and physical factors. Assessing which factors affecting MBR permeate quality is difficult because of complex reaction mechanisms that vary with both time and physical attributes (environmental conditions) of the system. Given the nonlinearity, uncertainty, and dynamic features of MBR process, an alternative data analysis technique is needed. Artificial Neural Networks have ability to capture the relationships between multiple operational parameter variables and treated water quality (Côté *et al.* 1995, Lee *et al.* 2005) but they tends to be “black box” models which neither show dependencies between variables nor provide probabilistic predictions (Pittman 2008).

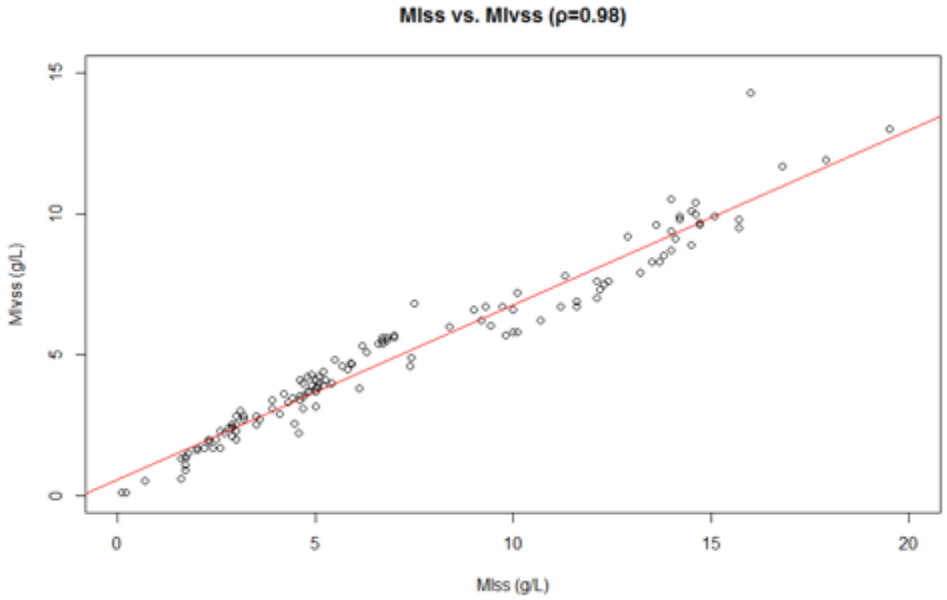

Figure 4 - MLSS vs. MLVSS of 315 data sets from 9 full-scale MBRs across Australia.

Over the last decade, BBN is increasingly used for modelling complex domain such as ecosystems and environmental management (Uusitalo 2007). A BBN is a probabilistic graphical model for reasoning under uncertainty, with a set of variables (or nodes) and directed arcs that describe the sets of conditional dependencies between variables (Pearl 2000, Korb *et al.* 2011). Based on these characteristics, BBN can offer several advantages including:

- Capability to model complex systems where there are multiple variables influencing each other;
- Ability to deal with uncertainty as the content of each variable is presented as probability distribution so BBN not only gives the result but also its expected frequency;
- Transparency, which provides opportunity to gain insights about the system as well as make it a good communication tool (Sahely *et al.* 2001);
- Ability to deal with missing data as algorithms in BBN can handle situations with missing observations which are often the case in environmental data;
- Capability to combine different sources of knowledge, e.g. expert knowledge regarding variables on which little or no data exist can be introduced as prior information to the net. These priors are then updated with real data to provide a synthesis of expert knowledge and real data. This synthesis can then be used as a prior in a new study (Uusitalo 2007);
- Bidirectional: the same network can be used without modification to diagnose causes to specific problems given information about the output variables or to predict increases in operational efficiency given information about the input variables (Sahely *et al.* 2001);
- Relatively easy to be modified and updated with new data and knowledge (Sahely *et al.* 2001).

Although, BBN offer a lot of benefits for modelling complex systems, their applications in wastewater treatment systems are still very limited. Bayesian analysis of MBR operating data in combination with LRV has not been attempted before. Accordingly, this study aims to develop and apply BBN to identify factors affecting LRV of microbial indicators through MBRs.

7.2.2 Constructing a Bayesian network for MBRs

The development of the MBR Bayesian net in this study follows the major steps in developing a BBN presented in Figure 5 below. Firstly, the objective and scope of the model need to be determined. Then the next step is to develop the model structure including defining nodes and connections between the nodes in the net. After that, the model is parametrised that includes defining states and intervals as well as filling the CPT table for each node. The final step is to evaluate and validate the model. More details about these steps are provided below.

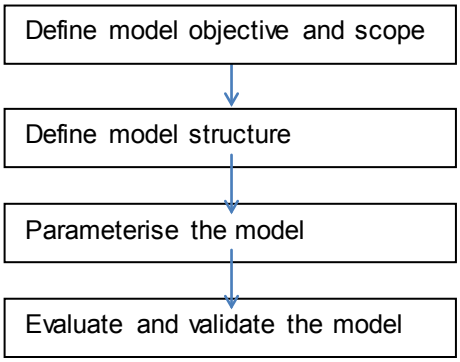

Figure 5 - Major steps in developing a BBN (adapted from Ticehurst et al. (2008) and Kraft (2009)).

7.2.2.1 Defining model objective and scope

As stated above, the objective of this study is to develop and apply BBN to identify factors affecting LRV of microbial indicators through MBRs.

7.2.2.2 Defining model structure

In this step, variables (nodes) and connections between the variables in the model are determined.

Selecting variables for the model

The variables in BBN should be controllable, predictable or observable (Borsuk *et al.* 2004, Chen *et al.* 2012). Insignificant variables should not be included as this increases the complexity of the network and reduces the sensitivity of the model outputs to important variables (Chen *et al.* 2012). In this study, the selection of variables for the MBR BBN was conducted considering literature on key membrane design and operating parameters (Judd *et al.* 2011), the Victorian guidelines for validation of MBRs (VDoH 2013), previous validation reports of full-scale MBR plants, as well as data available for model evaluation and validation. As presented in Section 7.2.1, MLSS and MLVSS data are linearly correlated with $\rho = 0.98$, so only one of the two parameters needs to be included in the model. MLSS was selected because it is quicker, easier to analyse offline, and it can also be monitored online. The selected variables and range of data available for these variables are presented in Table 22.

LRV indicator was calculated from influent indicator density and permeate indicator density. As indigenous influent indicator density cannot be controlled, given a fixed influent indicator density, factors that potentially cause an increase in the likelihood of higher permeate indicator density were equivalent to causes of low LRV. This approach was applied throughout this study in determining factors influencing LRV indicators.

Defining connections between variables

Structure of BBN can be developed based on expert knowledge or automatic structure learning. Literature have shown that environmental processes, which often includes a lot of variation and uncertainty, cannot be completely accurately estimated based on available data (Uusitalo 2007, Chen *et al.* 2012). However, where the expert knowledge on the system is incomplete, structure learning process provides a new perspective on the problem, a better appreciation of the complexity of the system, and a better understanding of the system and the limitations of our data (Alameddine *et al.* 2011). In this study, automatic structure learning was conducted using R software (R-project 2014) to provide better insight about the system and the limitation of the data. Then, the structure of the net was developed based on expert knowledge through an iterative process during a series of workshops between experts of SP1 and SP4. The net was constructed in Netica™ Bayesian modelling software (Norsys 2015). Netica™ provides a popular and simple graphical interface for building and working with BBNs (Norsys 2015).

Table 22 - Selected variables for the MBR BBN and range of data available for these variables.

Group of variables	Variables (nodes)	Unit	Data range[1] (Low, IQR, High)
Reactor variables	Solid retention time (SRT)	d	12, 32-126, 147
	MLSS	g/L	0.1, 3.4-13, 20
	Hydraulic retention time (HRT)	h	4, 17-39, 100
	Dissolved oxygen (DO)	mg/L	0, 1.5-4.8, 8.3
	Temperature in mixed liquor	°C	16, 21-25, 30
Membrane conditions	Flux	LMH	0.4, 5.2-22, 37
	Transmembrane pressure (TMP)	Kpa	0.4, 5.9-6.9, 50
	Permeability	LMH/Kpa	0.1, 0.8-5.1, 33
	Membrane age	months	1.0, 5.0-27, 217
	Membrane pore size	µm	0.04-0.4
	Membrane configuration		
	Chemical cleaning type		
	Time after chemical cleaning	h	
Bulk quality parameters	Influent dissolved organic carbon (DOC)	mg/L	9.0, 61-88, 182
	Mixed liquor DOC	mg/L	4.6, 10-21, 77
	Permeate DOC	mg/L	4.9, 7.4-12, 932
	Permeate turbidity	NTU	0.01, 0.03-0.13, 3.7
	Influent pH		6.3, 7.5-8.2, 10
	Mixed liquor pH		3.8, 6.9-7.7, 9.0
	Permeate pH		3.0, 6.9-7.7, 9.0
	Capillary suction time (CST)	s	11, 22-44, 274
Microbial indicator densities	Log Somatic influent		1.6, 5.0-5.5, 7.4
	Log Somatic mixed liquor		2.0, 4.6-5.6, 6.7
	Log Somatic permeate		1.0, 1.0-1.7, 3.1
	Log FRNA influent		3.0, 5.0-6.0, 7.0
	Log FRNA mixed liquor		2.0, 4.0-5.0, 6.0
	Log FRNA permeate		1.0, 1.0, 2.0
	Log *E. coli* influent		4.3, 6.6-7.1, 9.4
	Log *E. coli* mixed liquor		3.3, 5.5-6.3, 8.6
	Log *E. coli* permeate		0.0, 0.0-0.5, 2.2
	Log Perfringen influent		3.5, 5.2-5.6, 7.1
	Log Perfringen mixed liquor		5.8, 6.5-6.9, 7.4
	Log Perfringen permeate		0, 0, 2.6
Calculated LRV microbial indicators	LRV Somatic		Calculated from influent and permeate densities
	LRV FRNA		Calculated from influent and permeate densities
	LRV *E. coli*		Calculated from influent and permeate densities
	LRV Perfringen		Calculated from influent and permeate densities

[1]IQR = Interquartile range, Low = lowest and High = highest, of parameters from Full Scale site sampling.

7.2.2.3 Parameterising the model

This step includes defining states and intervals for each node in the net. The more states, the more data are needed to fill the CPT table. In practice, data often are not large enough to allow high numbers of intervals per variable. Therefore, in order to build a meaningful BBN, the numbers of states are often restricted (Uusitalo 2007). In this study, due to limited variability in the available data, 2 states were selected for each node in the net to minimise empty probability in the CPT table. The intervals were defined by automatic discretisation with equal-frequency method in Netica™ interface.

7.2.2.4 Evaluate and validate the model

10-fold cross validation method was used to validate the model using the R software (R-project 2014). This approach first partitioned the data into 10 equally sized sets and then used 9 of these partitions for parameter learning and the remaining for holdout testing (Koller *et al.* 2009). This was repeated 10 times in order to test 10 partitions of the dataset. This cross validation was conducted using the package RNetica in R software (Almond R. 2014). RNetica provides an R software interface to Netica™ including the same functionalities as the Netica™ software. The script was designed to perform K-fold cross validation.
In this study, the area under the receiver operating characteristic (ROC) curve was used to assess the accuracy of the model. The ROC curve is a graphical plot that illustrates the performance of a binary classifier system as its discrimination threshold is varied. The curve is created by plotting the true positive rate against the false positive rate at various threshold settings. The area under the ROC curve (AUC) is widely used to evaluate the accuracy of classification tests (Flach *et al.* 2011). An area of 1 represents a perfect test and an area of 0.5 represents a worthless test.

7.2.3 Identifying factors affecting LRV of microbial indicators by MBRs

As stated in Section 7.2.2.2, LRV indicator was calculated from influent indicator density and permeate indicator density. As indigenous influent indicator density cannot be controlled, factors effecting LRV of microbial indicators were considered to be equal to factors causing an increase in the likelihood of higher permeate indicator density given the same influent density.

Factors affecting log permeate indicator density were determined by considering the importance of the factor in predicting log permeate indicator density. Firstly, the AUC score for predicting log permeate indicator density when data of all other variables in the net available was determined and used as a baseline AUC. Then, the data of one variable in the net was removed, and the AUC score for predicting log permeate indicator density was calculated. This step was repeated for all other variables in the net. After that, the AUC scores in the absence of data of each variable were compared with the baseline AUC. The variable, without its data, the AUC score reduces more than 1% compared to the baseline AUC, was considered as important factor influencing the log permeate indicator density. In all cases, each AUC score was calculated with 3 times with 3 different seeding ratios and the mean of these 3 calculations was used.

7.3 Findings from the preliminary MBR Bayesian Network

MBRs feature a large number of, potentially interrelated, factors that could contribute to LRV. In order to identify significant factors, a Bayesian network was constructed, in conjunction with SP4 Multiple Barriers, to assess impact on indicator LRV. If a factor could be found significant, the effect of increasing or decreasing that factor could then be presented in terms of likely influence on LRV.

The MBR Bayesian network was trained on over 100 site visits worth of data. Node connections were informed through an iterative process, incorporating expert knowledge workshops and automated structure learning.

Indigenous influent indicator densities cannot be controlled; as a result, calculation of LRV can often relate in censored ‘greater than’ LRVs, where indicators are removed below the limit of detection. In order to circumvent this problem, the Bayesian network was interrogated

to ascertain factors that, when changed, would increase the likelihood of higher activated sludge and permeate indicator densities. Given that influent densities were fixed in the model (according to the complete set of site visit data), factors that lead to high permeate indicator densities were equivalent to causes of low LRV. The generalized approach for use of a Bayesian network to identify effect and significance of influencing factors is illustrated in Figure 6.

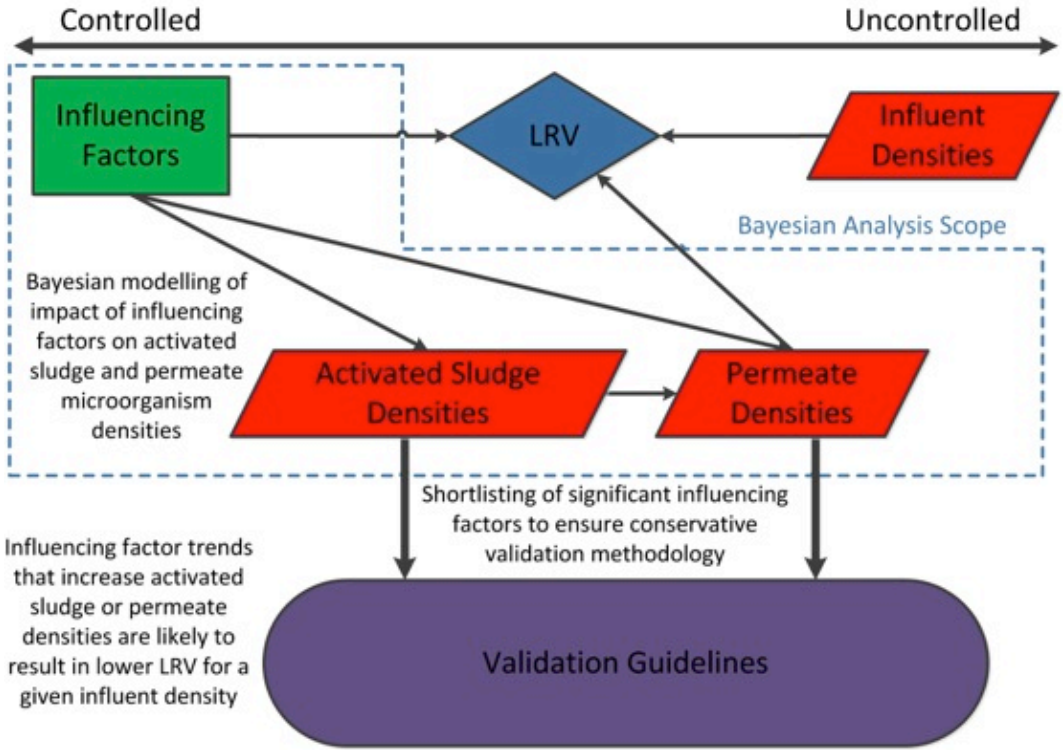

Figure 6 – A Bayesian approach to identification of significance and effect of influencing parameters on LRV and integration in the validation guidelines.

Factors that could affect or relate to LRV were shortlisted in Table 23 from review of MBR design literature (Judd 2011), the Victorian validation guidelines (VDoH 2013) and previous validation reports (Appendices A and C). Factors were classified into types, according to when the parameters could be chosen, determined or changed. Factor types classification included:

- Design: Factors chosen at the design stage,
- Operational Control: Factors that could be altered at operation,
- Operational Measurement: Simple factors that are measured during operation but are controlled indirectly (eg can reduce TMP by lowering flux), and
- Water Quality/Measured Parameters: Factors that are measured but may not be possible to control directly.

Preliminary Bayesian analysis of MBR influencing factors suggested that operation under the following conditions resulted in a higher likelihood of a lower LRV:

- Low HRT
- High flux
- Low TMP
- High permeability
- Low MLSS
- High permeate turbidity and
- High dissolved oxygen in biomass

The inclusion of flux, TMP and permeability is under review, as these parameters are not wholly independent. The presence of high dissolved oxygen cannot be explained and is under further investigation (e.g. as it could be related to increased level of shear provided to the biomass flocs, potentially damaging them).

pH was investigated but did not yield significant changes in LRV. SRT influenced MLSS/MLVSS concentrations but did not influence the indicator densities in mixed liquor and permeate directly. DOC did not influence the indicator densities in mixed liquor and permeate directly, but changes in DOC in mixed liquor and permeate follow the same trends as changes in indicator densities in these samples, indicating that DOC is a potential surrogate for these indicators in mixed liquor and permeate. Similarly, CST did not directly influence indicator densities in mixed liquor and permeate. Analysis of membrane pore size suggested lower LRV at higher pore size. However, membrane pore size, material and configuration was not included in the major trends as regardless of the starting pore size, each membrane type will need to satisfy the same validation methodology. In addition, only 1 of 10 sites featured a higher range pore size (0.4 µm, flatsheet), as a result there maybe bias due to the sample set.

Temperature in mixed liquor was initially included for Bayesian analysis but was removed, in order to simplify the network, after not demonstrating significant sensitivity to any other variables in the net. Temperature is still under investigation in further MBR Bayesian Network revisions. Membrane ageing resulted in an increased likelihood of higher virus LRV, but resulted in lower bacteria LRV. Accordingly, the membrane ageing relationship has been listed as uncertain and will be monitored on future revisions of the Bayesian network.

The full set of shortlisted factors including range of data analysed, significance and trends with LRV is summarised in Table 23.

SUMMARY: LRV indicators are affected by log indicator influent and log indicator permeate. Table 24 presents potential operational parameters influencing log indicator ML and log indicator permeate.

Table 23 - Shortlisted influencing factors for MBR LRV and outcomes of the Bayesian modelling

Factor (units)	Factor Type[i]	Low, IQR, High[j]	Candidate in Bayes Net	Factor Range for Conservative LRV[k]	Comments
SRT (d)	C	12, 32 – 126, 147	Yes	No influence	
HRT (hr)	C	4, 17 – 39, 100	Yes	Low	
F/M ratio (gBOD/gMLVSS/d)	C/Q	0.02, 0.02 – 0.04, 0.06	No	N/A	Investigated by use of DOC as surrogate for organic matter concentrations
Flux (LMH)	C	0.4, 5 – 22, 37	Yes	High	
TMP (kPa)	M	0.4, 5 – 7, 50	Yes	Low	
Permeability (LMH/kPa)	M	0.1, 0.8 – 5.1 , 33	Yes	High	Quantifies current membrane fouling state
Membrane Type[l]	D	N/A	N/A	N/A	Same final performance testing requirement
Pore size (µm)	D	0.04 - 0.4	Yes	High	Most 0.04 µm.
Membrane Age (months)	M	1, 5 – 27, 217	Yes	Uncertain	To inform on revalidation conditions. Removal variability increased with age. Different effect for virus and bacteria.
Membrane Aeration	C	N/A	No	N/A	Used DO as a surrogate measured parameter
Membrane Area	D	N/A	No	N/A	
Chemical Cleaning[m]	C	N/A	No	N/A	Permeability as surrogate for fouling condition
Chemical Dosing[n]	C	N/A	No	N/A	Could be investigated qualitatively from site knowledge
MLSS/MLVSS (g/L)	Q	0.1, 3.4 – 12.9, 20	Yes	Low	Except for CP as low MLSS implies high wasting rate
CST (s)	Q	11, 22 – 44, 274	Yes	No influence	
COD (mg/L)	Q	268, 282 – 530, 2230	No	N/A	DOC as surrogate for organic matter concentrations
BOD (mg/L)	Q	60, 127 – 195, 353	No	N/A	DOC as surrogate for organic matter concentrations
DOC (mg/L)	Q	9, 61- 88, 182	Yes	No influence	
Ammonia (mg/L)	Q	7, 35 – 50, 95	No	N/A	Small data set
Turbidity (NTU)	M	0.01, 0.03 – 0.13, 3.7	Yes	High	Minimal high turbidities make correlation of turbidity poor
pH	C/M	3.8, 6.9 – 7.5, 9.0	Yes	No influence	
DO (mg/L)	M	0, 1.5 – 4.8, 8.3	Yes	High	
Temperature (°C)	M	16, 21 – 25, 30	No	N/A	Low sensitivity to findings

[i] D = Design, C = Operational Control , M = Operational measurement, Q = Water Quality/offline measured parameter

[j] IQR = Interquartile range, Low = lowest and High = highest, of parameters from SP1 Full Scale Site Sampling

[k] If factor range is high means that for high levels of that factor the likelihood of a low LRV increases. No correlation indicates no discernable trend for range tested

[l] Includes membrane material and membrane configuration

[m] Includes chemical type, cleaning duration and mode (eg CIP or CEB, backwash or manual cleaning).

[n] Includes pH adjustment, coagulant addition and possible feedwater BOD supplement.

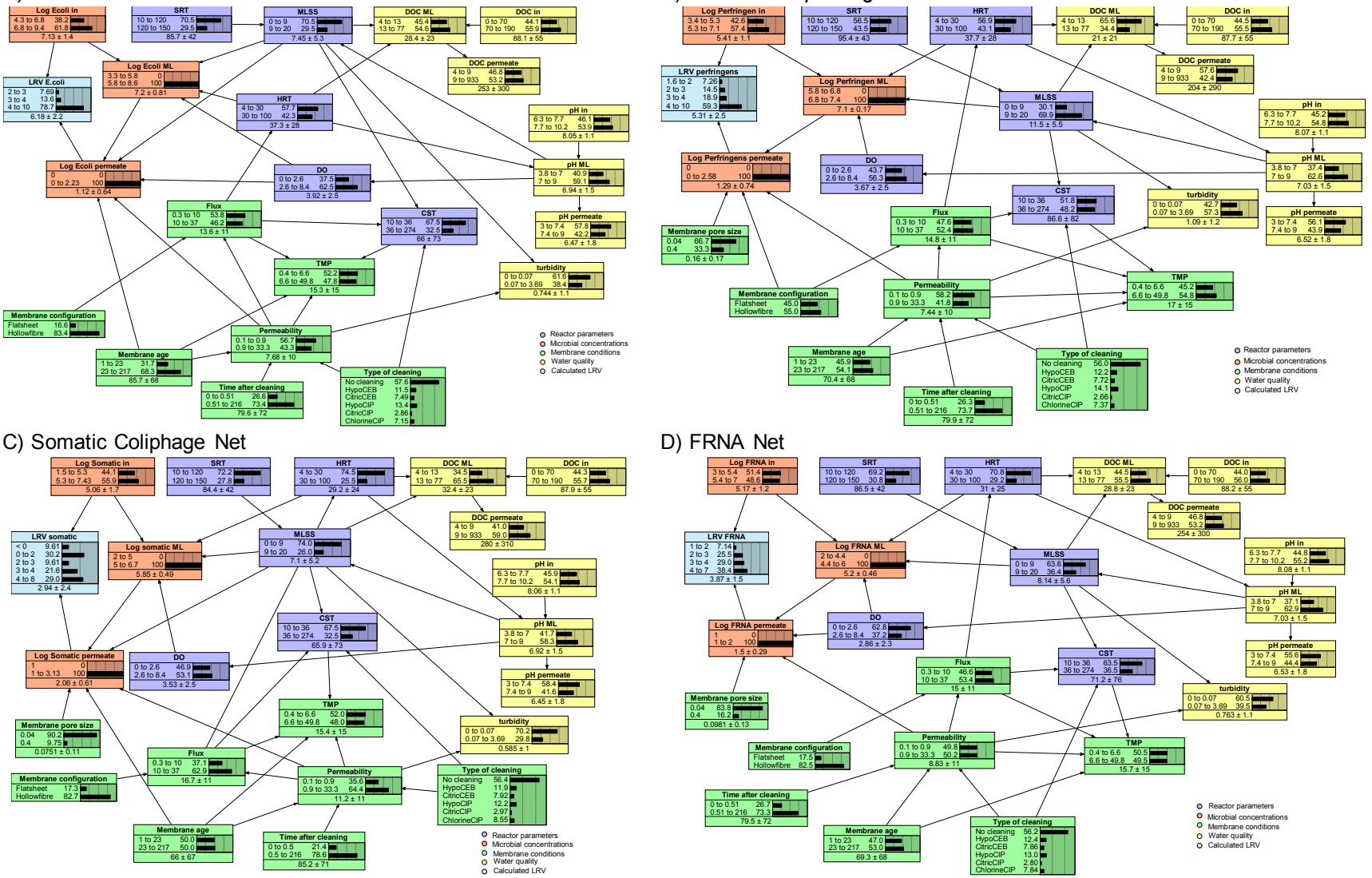

Figure 7 – Indicator specific Bayesian Networks used to draw conclusions on significant factors influencing MBR LRV

Table 24 - Potential operational parameters influencing log indicator ML and log indicator permeate

Indicator node	MLSS (0.1 to 19.5 mg/L)	HRT (4.5 to 99.9 h)	DO (0.0 to 8.3 mg/L)	Permeability (0.1 to 33.3 LMH/KPa)	Membrane age (1 to 217 month)	Membrane pore size (0.04 µm and 0.4 µm)
Log *E. coli* ML (↑) (3.3 to 8.6)	Yes direct (↓) *Data: 254 (251)*[15]	Yes direct (↓) *Data: 233 (232)*	Yes direct (↑) *Data: 219 (216)*	No	No	No
Log *E. coli* permeate (↑) (0.0 to 2.3)	Yes direct (↓clear) *Data: 253 (101)*	Yes indirect through log *E. coli* ML (↓) *Data: 232 (87)*	Yes direct (↑) *Data: 218 (90)*	Yes direct (↓?) *Data: 266 (93)*	Yes direct (↑clear) *Data: 306 (102)*	No
Log Somatic ML (↑) (2.0 to 6.7)	Yes direct (↓) *Data: 188 (186)*	Yes direct (↓) *Data: 171 (170)*	Yes direct (↑) *Data: 173 (172)*	No	No	No
Log Somatic permeate (↑) (1.0 to 3.1)	Yes direct (↓) *Data: 187 (70)*	Yes indirect through log Somatic ML (↓) *Data: 170 (62)*	Yes indirect through log Somatic ML (↑) *Data: 172 (65)*	Yes (↑ clear) *Data: 203 (89)*	Yes direct (↓) *Data: 224 (93)*	Yes direct (↑) *Data: 229 (98)*
Log FRNA ML (↑) (2.0 to 5.9)	Yes direct (↓ weak) *Data: 195 (190)*	Yes direct (↓clear) *Data: 179 (175)*	Yes direct (↑clear) *Data: 180 (176)*	No	No	No
Log FRNA permeate (↑) (1.0 to 2.2)	Yes indirect through log FRNA ML (↓ weak) *Data: 195 (11)*	Yes indirect through log FRNA ML (↓ weak) *Data: 179 (11)*	Yes indirect (↓?weak) *Data: 180 (9)*	Yes direct (↑) *Data: 190 (11)*	Uncertain *Data: 207 (11)*	Yes direct (↑) *Data: 212 (11)*
Log Perfringen ML (↑) (5.8 to 7.4)	Yes direct (↑clear) *Data: 253 (253)*	Yes direct (↓) *Data: 232 (232)*	Yes direct (↑) *Data: 216 (216)*	No	No	No
Log Perfringen permeate (↑) (0.0 to 2.6)	Yes indirect through log Perfringen ML (↑ weak) *Data: 253 (20)*	Yes through log Perfringen ML (↓ weak) *Data: 232 (19)*	Yes indirect through log Perfringen ML (↑ weak) *Data: 216 (19)*	Yes direct (↓?) *Data: 265 (19)*	Uncertain *Data: 305 (21)*	Yes direct (↑) *Data: 300 (21)*

(Combination assessment results show that **Flux** and **TMP** are the two additional potential factors influencing Log indicator permeate)

[15] *Data: 254 (251): mean there are 254 data sets having both MLSS and Log E.coli ML, among these 254 data sets, 251 have Log E.coli ML>LOD and 3 have Log E.coli ML≤LOD*

8 Chemical cleaning impact on LRV

8.1 Introduction

In water recycling applications, a thorough understanding of pathogen removal performance and variability, for each unit operation, is imperative. Validation is a process to confirm that a treatment technology can, and will continuously, meet specified performance targets. Any event that compromises the pathogen removal efficiency must be detected and quantified to inform appropriate corrective action (Trinh *et al.* 2014). The primary mechanisms for pathogen removal in a membrane bioreactor (MBR) are size exclusion, entrainment within activated sludge flocs or membrane fouling layer, and biological predation. Previous studies have indicated the importance of the fouling layer in aiding removal of viruses, that are smaller than the membrane pore size (Hai *et al.* 2014). Consequently, removal of this fouling layer following chemical cleaning, was identified as a key short-term event requiring quantification at full scale.

Multiple chemical cleaning cycles, over the membrane life, are expected to result in membrane chemistry and morphology changes (Arkhangelsky *et al.* 2007, Puspitasari *et al.* 2010, Wang *et al.* 2010), with consequences for pathogen rejection (van den Akker *et al.* 2014). To date, very few investigations have been performed on full scale MBRs accounting for change in log removal value (LRV) due to extended service life. As part of the Australian Water Recycling Centre of Excellence (AWRCoE) project, "Establishement of a National Validation Framework for water recycling" (NatVal), an MBR sub-project has been established to address knowledge gaps associated with MBR validation.

In this study, a full-scale MBR was monitored over a period exceeding 6 months. At the time of monitoring, the MBR had been in operation for two years on municipal wastewater. During the monitoring period, influent, activated sludge, and permeate samples were taken to quantify LRV. Sampling was conducted before and after weekly maintenance backwashes of sodium hypochlorite (NaOCl). Additionally, a yearly, offline, clean in place (CIP) was observed. Indicator organisms tested included somatic coliphage (SC), FRNA bacteriophage (FRNA), *E. coli* (EC), total coliforms (TC), and *Clostridium perfringens* (CP).

Previous studies already concluded that the effect of NaOCl cleaning on MBR LRVs were negligible (Hirani *et al.* 2014, van den Akker *et al.* 2014). However, this work is the first study to assess two different cleaning methods (CIP and chemically enhanced backwashes (CEB)), on the same full scale site with reference to the site operating data, and to report permeate chlorine residual resulting from cleans.

8.2 Method

8.2.1 MBR Description

A multiple barrier process at Pitt Town Local Water Centre (PTLWC), N.S.W, was commissioned in May 2012 (Figure 8). PTLWC receives domestic wastewater from a rising main as part of the infrastructure for a dual reticulation scheme, for a new housing development. A MBR was installed as part of the PTLWC to ensure biological treatment and first stage disinfection. The MBR features anoxic (40%), aerobic (51%) and membrane compartments (9%) with a total working volume of 97 m^3. Hollow fibre ultrafiltration membranes with a total area of 558 m^2 and nominal pore size of 0.04 µm were installed. Typical operating flux and mixed liquor suspended solids (MLSS) for the monitoring period were 6 $L.m^2.h^{-1}$ and 8000 – 14000 $mg.L^{-1}$ respectively. Sludge was wasted in 10,000 L batches via tanker, at the discretion of the operator, resulting in an average solids retention time of 100 days. The critical control point for the MBR was permeate turbidity. If turbidity exceeded 0.2 NTU, the permeate was bypassed to the influent balance tank, until turbidity returned and remained below 0.2 NTU for 1 min. Chemical dosages included acetic acid, to increase influent BOD, sodium hydroxide, to normalise pH and aluminium sulphate for phosphorous removal. Alum and acetic acid additions were programmed at a predetermined rate into the anoxic zone. Sodium hydroxide additions were inline, controlled via pH.

Figure 8 - The water recycling plant at Pitt Town Local Water Centre

8.2.2 Chemical Cleaning Regimes

A chemically enhanced backwash (CEB), performed once per week, consisted of 8 cycles of the following sequential steps; aeration (400 s), sodium hypochlorite (NaOCl) pulse (20 s), backflush (60 s) and soak (240 s). The CEB regime resulted in a NaOCl concentration of 100 – 300 $mg.L^{-1}$ flushed in reverse through the membrane.

A yearly CIP involved the following steps: isolation of the activated sludge compartments, drain down of the membrane compartment, soaking the membranes in 1000 $mg.L^{-1}$ NaOCl overnight, discharge of the cleaning solution and refill of the membrane compartment with activated sludge. The balance volume (9%) was made up with influent waste water. The MBR was then returned to service.

8.2.3 Sampling and Control Data

For each sampling event, grab samples were taken from influent wastewater, mixed liquor (recycled activated sludge line) and permeate (before UV disinfection). Four permeate samples were collected during the CEB events. Two control permeate samples were taken before cleaning, one immediately after cleaning and one 2 hr after cleaning. For CIP, influent, mixed liquor and duplicate permeate grab samples were taken immediately upon restart and then daily, excluding weekends, for 6 days following the cleaning. Additional control samples of influent, mixed liquor and permeate were taken randomly over the 6 month period.

In order to define event significance, a control charting approach was implemented. Data obtained during the monitoring period was organized into subgroups according to whether it was normal operation or not. Normal operation was defined as samples taken before cleaning, as well as discussions with operators about recent events in between sampling visits. If recent operational events, such as weather or maintenance shut downs, were deemed to potentially bias normal operating conditions, data was excluded from the control set. Monte Carlo simulation and probability density function (PDF) fitting were used with all control data, to characterize microbial removal and variability (Figure 9), in order to arrive at a statistically significant benchmark. To this end, lognormal PDFs were fit to cumulative microorganism densities, with goodness of fit analysed by root mean squared error. A LRV distribution was then calculated using the influent and permeate microorganism PDFs, via Monte Carlo simulation with @Risk software (Palisade Corporation, version 6.0) and Latin Hypercube sampling (using 10,000 iterations). Previous studies have used similar approaches in order to address limitations due to concentrations below permeate limits of detection (LOD) and to adequately account for system variability (Olivieri *et al.* 1999, Khan *et al.* 2010, van den Akker *et al.* 2014). Lognormal distributions were previously shown as adequate for modelling parameters in treated and untreated wastewater (Oliveira *et al.* 2012).

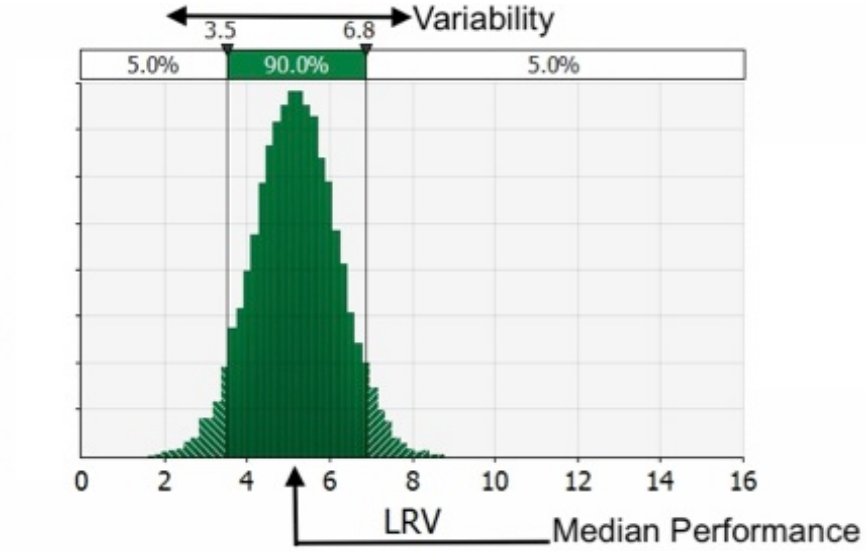

Figure 9 - Expressing log removal value as a distribution

8.2.4 Microbial Indicator Analysis

Two virus indicators, somatic coliphage (SC) and FRNA bacteriophage (FRNA), along with two bacteria indicators, *E. coli* (EC) and total coliforms (TC) and one protozoan indicator *Clostridium perfringens* (CP) were analysed in this study. The SC and FRNA methods were not established in time to adequately quantify the effect of CIP, but were analysed around CEB. Brilliance agar (Oxoid CM1046) was used to enumerate both EC and TC, which were incubated at 37 °C for 24 hr. TC were enumerated by counting both purple and pink colonies that were visible on the agar, while the number of presumptive EC was obtained by only counting the purple colonies. CP were enumerated using the tryptose sulphite cycloserine agar for CP (Oxoid CM0587), and incubated anaerobically at 37 °C for 24 hr. FRNA were quantified using the double agar layer (DAL) technique according to previously published methods (Noble *et al.* 2004), using *E. coli* F-amp (ATCC #700891) as the host and MS2 bacteriophage (ATCC #15597-B1) as the positive control. SC were also analysed by the DAL technique with *E. coli* CN-13 (ATCC #700609) as the host and Phi X174 (ATCC # 13706-B1) as the positive control. All bacterial indicators measured within the permeate were quantified using membrane filtration (Method 9215D, (APHA 1992)), whereby a desired volume of sample (typically 5, 50 and 100 mL) was filtered through a 47 mm diameter, 0.45 µm gridded filter membrane (Millipore, S-Pak, type HA). The filter membrane was then transferred onto the surface of a plate of selective agar. Data was reported in colony forming units (CFU) for bacterial indicators and plaque forming units (PFU) for phage per 100 mL volume of sample. For SC and FRNA, LOD was 10 PFU per 100 mL. For CP, EC and TC, LOD was 1 CFU per 100 mL.

LRV was calculated for each microbial indicator using Equation 6.

$$LRV_X = \log_{10}\left|\frac{C_{In}}{C_{Permeate}}\right| \qquad \text{Equation 6}$$

Where C_{In} and $C_{Permeate}$ were the densities of microbial indicator X, from paired grab samples, analysed on influent and permeate respectively. X being one of the indicator organisms (e.g. LRV_{TC} was the log removal of total coliforms).

8.2.5 Bulk Parameters and Operational Data

During cleaning events, permeate turbidity was recorded online with a HACH FT660 Laser nephelometer. The online reading at the time of sampling was recorded as an instantaneous point, representative of the sample. Permeate flow rate and trans-membrane pressure (TMP) were retrieved from site SCADA systems, for the sampling events. Total chlorine was measured on the permeate using a HACH pocket chlorimeter and DPD reagent pillows (HACH Method 8167). Total chlorine was tested before, and after chemical cleaning events at 5 min intervals.

8.3 Results and Discussion

8.3.1 Normal System Variability

Over the 6-month sampling period, 28 paired, influent and permeate, samples were taken for bacteria and protozoan indicators and 14 for virus indicators. CP, EC, TC, FRNA and SC were detected, at or above the limit of detection (LOD), in the permeate for 26, 66, 100, 5 and 20% respectively, for all samples satisfying normal operating criteria (Table 25).

Table 25 - Number and location of control samples analysed over 6 months. Brackets indicate samples with microorganism densities below the LOD.

Indicator	Sample Location		
	Influent	Mixed Liquor	Permeate
Clostridium perfringens	28	25	9 (25)
E. coli	28	25	23 (12)
Total Coliforms	28	25	33
FRNA Bacteriophage	14	11	1 (19)
Somatic Coliphage	14	11	4 (16)

Establishment of a control distribution for FRNA was not possible, as this indicator was only detected on one occasion in the permeate, at the LOD. Accordingly, the lowest LRV, calculated with the permeate LOD, was chosen as the lower control limit (LCL) i.e. LCL was $LRV_{FRNA} > 3.7$. Control distributions were created for all other indicators. 5th percentile LRVs from distributions were defined as the LCL. If an LRV were to fall below the 5th percentile then a significant deviation with respect to 'normal' operation had occurred. The 5th percentile LRVs for CP, EC, TC and SC were 5.0, 6.0, 5.9 and 3.9 respectively (Table 26). The results from this study were in agreement with LRVs reported for other full scale MBRs (Pettigrew *et al.* 2010, Marti *et al.* 2011, van den Akker *et al.* 2014). The use of Monte Carlo simulation to calculate control LRVs was advantageous, with LRV expressed as a distribution summarising not only performance, but also expected variability (Figure 9). Additionally, it was still possible to calculate a representative LRV, even though up to 80% of permeate readings were below the LOD.

Table 26 - LRV distribution parameters representing normal operational performance and variability of the MBR during the sampling period.

Indicator	LRV Distribution Parameter			
	5th %ile	Median	95th %ile	St.Dev
Clostridium perfringens	5.0	5.7	6.4	0.4
E. coli	6.0	6.7	7.3	0.4
Total Coliforms	5.9	6.6	7.3	0.5
FRNA Bacteriophage	> 3.7	N/A	N/A	N/A
Somatic Coliphage	3.9	4.9	6.0	0.6

Based on a majority of observations, the MBR at PTLWC could be expected to exceed an LRV of 4 for bacteria and protozoa, and for viruses, an LRV of > 3.7 could be achieved (Table 2). Only one state-based validation guideline exists in Australia, published by the Victorian Department of Health in 2013. In order to encourage the use of multiple barriers in a water recycling scheme, no single unit operation can attain a log removal credit greater than 4 for virus, bacteria or protozoa (VDoH 2013). With respect to a maximum log removal credit of 4, the MBR in this study performed very well, under normal operating conditions.

8.3.2 Effect of Clean in Place on LRV

After restart of the MBR, following the CIP, levels of total chlorine in the permeate were low, starting at 0.9 and dropping to 0.03 mg.L^{-1} within 30 min; indicating minor transfer of NaOCl across the membrane during the soak. As such, the permeate sample taken 1 hour after CIP was not affected by disinfectant residual. Membrane permeability was assessed by observation of SCADA flow and TMP data before and after CIP as 1.4±0.3 and 2.0±0.4 L.m^{-2}.h^{-1}.kPa^{-1} respectively. The slight increase in permeability indicated the CIP removed some portion of the accumulated membrane fouling. Instantaneous turbidity results were recorded from the onsite turbidity meter upon permeate sampling.

Grab samples were analysed for indicators at 1, 24, 48, 120 and 144 hr after the CIP. Upon start up, turbidity immediately spiked to 0.5 NTU, receding to 0.32 NTU at 1 hr. At 4 hr, turbidity had decreased to the typical value of 0.08 NTU. Some minor spikes in online turbidity to 0.1 NTU were evident at 18 and 42 hr, although not exceeding the critical control limit. LRV_{EC} slightly decreased to 5.2 – 5.4 during the two days following the CIP. After 120 hr, LRV_{EC} had recovered to 6.8. LRV_{TC} rose from 4.9 to 5.9 over the first 48hr after the CIP. After 120 hr, LRV_{TC} recovered, within the control limits, to 6.3. LRV_{CP} remained > 5.1 and was not detected in any permeate samples post CIP (Figure 10).

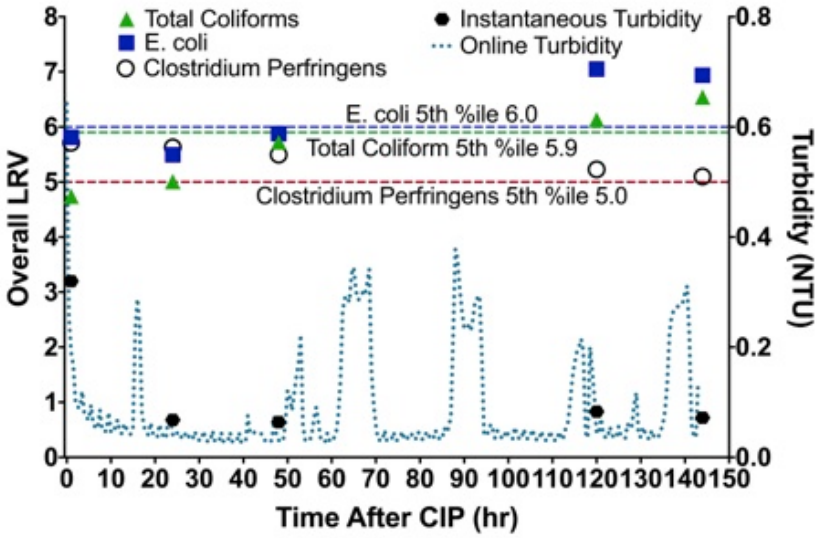

Figure 10 - LRV for TC, EC, CP and turbidity for 5 d following a CIP.

The significant breakthrough of TC for the first 48 hr following a CIP can be explained by the removal of fouling layer, acting as a shield for micro-defects on the membranes. Had defects become exposed as a result of CIP, the relatively high density of TC in the mixed liquor (4 – 8 x 10^7 CFU.100mL^{-1}) would have made passage across the membrane more likely, than for other indicators at lower densities. LRV_{EC} was below the 5th percentile, but not to the same extent as LRV_{TC}. Bacterial indicator recovery, with respect to the 5th percentile of the control set, occurred within 5 days.

8.3.3 Effect of Chemically Enhanced Backwash on LRV

CEB with NaOCl was observed for three weekly cleans. Up to 35 mg.L^{-1} total chlorine was observed in the permeate immediately upon system restart. After 20 min, chlorine residual returned to the LOD of 0.03 mg.L^{-1}. Turbidity spike following CEB with NaOCl was as high as 0.32 NTU, recovering to 0.08 NTU at 2 hr (Figure 11).

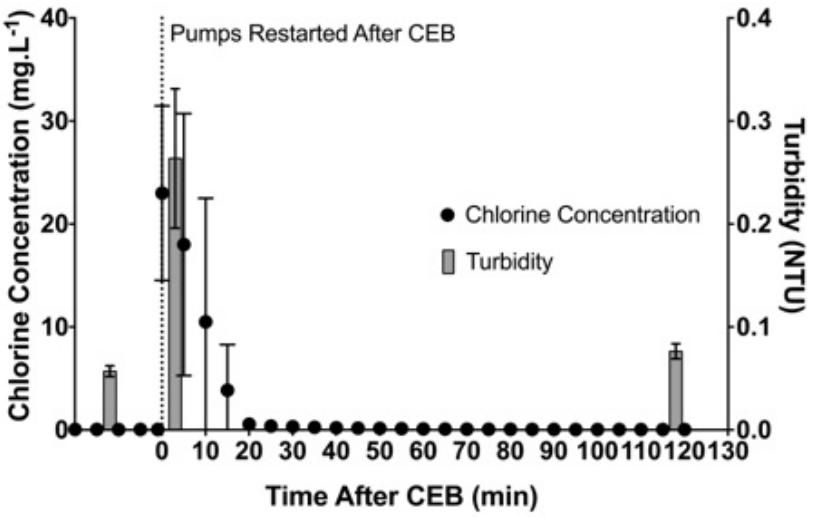

Figure 11 - Total chlorine and turbidity following a CEB. Error bars represent standard deviation from three CEB events.

Indicator organisms were assayed before, immediately after, and 2 hr after CEB. CP was not detected in any permeate samples during the trials, LRV_{CP} > 5.3 before and after CEB. LRV_{FRNA} varied between > 3.9 - > 5.4, dependent on influent densities, and was not detected in any permeate samples. LRV_{SC} was 4.6, detected at the permeate LOD for one trial, 2 hr after the CEB. For other trials, influent densities were only sufficient to yield LRV_{SC} of > 4.2 and > 4.3, unchanged by CEB. LRV_{EC} increased by 0.3 immediately after CEB, remaining high post clean, detected at the LOD in one of three trials, 2 hr after CEB. LRV_{TC} increased significantly from 7 to > 8.7, undetected immediately after cleaning. TC were detected in all three trials 2 hr after CEB, with an average LRV of 7.6 (Figure 12).

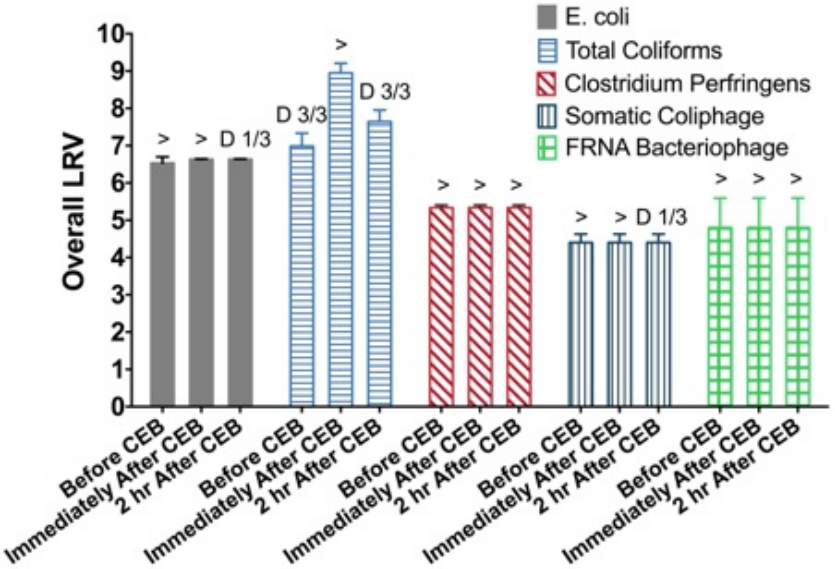

Figure 12 - LRV before and after CEB with NaOCl. Excess chlorine significantly improved TC LRV immediately following CEB. '>' symbols denote permeate concentrations below LOD. Fractions denote number of permeate trials at or above LOD.

The initial sudden increase in LRV_{TC} was likely due to the excessive disinfectant concentration (35 mg/L) measured immediately after CEB. LRV_{TC} was still higher 2 hr after CEB than before the clean, although no disinfectant residual was present. It is likely that some reduction of LRV_{TC} before CEB was observed due to TC growth and gradual detachment from permeate pipe work. NaOCl concentration between 100 – 300 $mg.L^{-1}$ in the permeate line during CEB is expected to result in destruction of accumulated total coliform growth. The slight net increase in LRV_{TC} of 0.6, 2 hr post clean, may be indicative of the level

to which growth can negatively affect overall LRV, on a system not performing frequent CEBs. Following CEB, no indicator organism LRV fell below the 5th percentile LRV of the control set. Even though a majority of indicator LRVs were censored, with permeate concentrations below LOD, CEB does not appear to have a significant negative effect on LRV for up to 2 hr after. CEB may have a slightly positive effect on LRV, due to removal of bacterial growth. The removal of biofilm growth present in the permeation line by CEB may explain turbidity spikes immediately after CEB.

8.4 Conclusions

CIP removed fouling from the membrane, as indicated by permeability increase, but did not appear to result in significant breakthrough of disinfectant into the permeate line. As a result, the use of an oxidising disinfectant in the CIP process did not contribute to LRV. For up to 48 hr after CIP, bacteria LRV was below the 5th percentile of the control set indicating a significant change, outside of normal variability. Within 5 days, bacteria LRV recovered and protozoan LRV was stable, unaffected by CIP.

In contrast to CIP, CEB resulted in appreciable disinfectant quantities remaining in the permeate for up to 20 min. Elevated disinfectant concentration immediately after CEB appeared to increase LRV, even though turbidity was out of specification. The large spike in turbidity may indicate suspended solids passage, through unshielded defects in the membrane, or sloughing of biofilm from within the permeate line. 2 hr after CEB, no indicator LRV was significantly affected relative to the 5th percentile. At 2 hr after CEB, total coliform LRV displayed a slight increase, relative to results before cleaning, likely due to removal of coliform growth within the permeate network.

9 Membrane ageing, replacement, and potential revalidation

9.1 Introduction

Previous research into UF membranes suggested a failure rate of one broken fibre per module per year installed (Gijsbertsen-Abrahamse *et al.* 2006). Causes of membrane failure can be grouped under chemical degradation, presence of foreign bodies, faulty installation and faulty membrane/module structure (Le-Clech 2010). Chemical degradation and the presence of foreign bodies are failure modes that would increase in likelihood with increased exposure, or installation time. Chemical cleaning is a common strategy to mitigate fouling in MBR. Sodium hypochlorite (NaOCl) is commonly used in chemical cleaning of membranes at contact dosages ranging from 1000 – 5000 mg/L up to 2 times per year. Additionally, some installations may enact weekly chemical backwashing at lower NaOCl dosages, 100 – 300 ppm (Le-Clech *et al.* 2005). In an accelerated ageing study of poly vinylidene difluoride (PVDF) membranes to NaOCl, increased exposure was shown to increase mean pore diameter and reduce material hydrophobicity (Puspitasari *et al.* 2010). Poly ethersulfone (PES) membranes also displayed reduction in hydrophobicity and became more brittle, according to tensile test results, upon increasing NaOCl contact time (Arkhangelsky *et al.* 2007).

MBR suppliers offer specific lifetime guarantees in the order of 3 – 8 years (Le-Clech 2010). Recently, predictions of membrane life have been made via different methods. From correlation of total membrane through-put with mechanical stability loss of the membrane-module bond, a lifetime of 6.4 years at a mean flux of 20 L m^{-2} h^{-1}, or 8.5 years at 15 L m^{-2} h^{-1} was proposed (Ayala *et al.* 2011). Through rigorous assessment of North American sales data for Zenon MBR membranes and classification of the type of sale (replacement or new), an empirical model yielded a membrane/module life of 8 years; it was also stated that most failures were attributed to early generation module issues, which were no longer observed with the later generation; hence, the 8 year estimate was likely conservative (Cote *et al.* 2012). Through extrapolation of permeability (i.e., production capacity) decline to an unacceptable level, a lifetime estimate of 8 – 10 years was made. The model was based on observation of GE Zenon membranes, for 9 years, at one full scale facility (Fenu *et al.* 2012).

Some concern over long term water safety may be valid due to the increasing likelihood of integrity failure as a membrane ages. The objective of this study was to assess the impact of extended operating life on LRV of MBRs. This study featured a unique comparison of micro-organism LRV at a single full scale installation, with 10 year old membranes and subsequent replacement with new membranes. This work presents significant results, as previous studies have been limited to accelerated ageing at a laboratory scale.

9.2 Method

9.2.1 Site description

Sampling was conducted at Sydney Water's North Head MBR. North Head is a primary sewage treatment plant situated in Manly, New South Wales, Australia. Average dry weather flow throughout the study was 310 ML/d. The MBR at North Head was commissioned in 2005 and designed to produce 2 ML/d of recycled water from screened and settled sewage, taken after the primary sedimentation tanks. The bioreactor is configured as a Modified Ludzack-Ettinger biological process. The bioreactor contains aerobic, anoxic and membrane compartments to a total volume of 450 m^3. Permeate from the MBR is chlorinated and used onsite for pump cooling water, screen and surface washdown, chemical dilution and firefighting. Originally the MBR was fitted with Memcor B10 membranes, with a total installed area of 3200 m^2. Due to declining hydraulic performances the decision was made to upgrade the plant with newer Memcor B40 membranes, increasing installed area to 4200 m^2.

9.2.2 Operational Parameters

Trans-membrane pressure (TMP) and permeate flowrate were recorded instantaneously on sampling times from the onsite SCADA system. Hydraulic retention time (HRT), membrane flux and permeability were calculated accordingly. Sludge wastage rate, from SCADA, was totalized each day for the sampling period and used to calculate solids retention time (SRT) in days until averaged over the month.

9.2.3 Sampling

Grab samples were taken of influent wastewater from the pipeline flowing into the bioreactor; activated sludge from the recycled activated sludge line and permeate before chlorination. Sampling was conducted over three periods, 1 month before replacement (22 sample days), immediately after replacement (21 sample days over 1 month and a follow up periods (16 samples 6 – 8 months after replacement). On two sampling occasions permeate results were omitted as NaOCl was detected in the permeate due to a leaking valve allowing passage of chemical cleaning solution.

9.2.4 Bulk water quality parameters

All samples were measured for pH and dissolved organic carbon (DOC). pH was determined with a BlueBox-pH meter from Instrument Works Pty Ltd. Influent and mixed liquor samples were centrifuged at 4400 rpm for 15 min and filtered through a 0.45 µm syringe prior to DOC analysis. DOC was analysed on a Shimadzu TOC-VCSH total organic carbon analyzer. DOC removal was assessed as a surrogate for biological performance of the bioreactor. Mixed liquor suspended solids (MLSS) and Mixed liquor volatile suspended solids (MLVSS) measurements were carried out on activated sludge samples, according to Standard Methods for Examination of Water and Wastewater (American Public Health Association/American Water Works Association/Water Environment Federation 2013). Capillary suction time (CST) was measured, as a surrogate for filterability, on activated sludge samples with a Triton Type 319 multipurpose CST.

9.2.5 Microbial Analysis

Two virus indicators, somatic coliphage (SC) and FRNA bacteriophage (FRNA), along with two bacteria indicators, *E. coli* (EC) and total coliforms (TC) and one protozoan indicator *Clostridium perfringens* (CP) were analysed in this study. The SC and FRNA methods were not established in time to adequately quantify the effect of CIP, but were analysed around CEB. Brilliance agar (Oxoid CM1046) was used to enumerate both EC and TC, which were incubated at 37 °C for 24 hr. TC were enumerated by counting both purple and pink colonies that were visible on the agar, while the number of presumptive EC was obtained by only counting the purple colonies. CP were enumerated using the tryptose sulphite cycloserine agar for CP (Oxoid CM0587), and incubated anaerobically at 37 °C for 24 hr. FRNA were quantified using the double agar layer (DAL) technique according to previously published methods (Noble *et al.* 2004), using *E. coli* F-amp (ATCC #700891) as the host and MS2 bacteriophage (ATCC #15597-B1) as the positive control. SC were also analysed by the DAL technique with *E. coli* CN-13 (ATCC #700609) as the host and Phi X174 (ATCC # 13706-B1) as the positive control. All bacterial indicators measured within the permeate were quantified using membrane filtration (Method 9215D, (APHA 1992)), whereby a desired volume of sample (typically 5, 50 and 100 mL) was filtered through a 47 mm diameter, 0.45 µm gridded filter membrane (Millipore, S-Pak, type HA). The filter membrane was then transferred onto the surface of a plate of selective agar. Data was reported in colony forming units (CFU) for bacterial indicators and plaque forming units (PFU) for phage per 100 mL volume of sample. For SC and FRNA, LOD was 10 PFU per 100 mL. For CP, EC and TC, LOD was 1 CFU per 100 mL.

9.2.6 Challenge Testing of Used Membrane Module

Upon replacement, one aged membrane module was taken for inspection and challenge testing. The challenge testing was conducted under clean water conditions over two different fluxes (10 and 30 $L/m^2/h$). pH was maintained from 7 – 8 by addition of either NaOH or HCl. For the first challenge test, MS2 stock solution was spiked into a 200L tank containing the

module and reverse osmosis water. For the second challenge test raw sewage was spiked into the challenge test tank in order to measure clean water rejection of indigenous EC CP, FRNA and SC. Pressure decay testing was also conducted on the module at starting pressures of 30 and 100 kPa according to the method outlined in the USEPA Membrane Filtration Guidance Manual (USEPA 2005).

9.2.7 Calculation of LRV and Monte Carlo Simulation

Three different LRV were calculated, LRV_{MBR} (Equation 7), LRV_{Bio} (Equation 8) and LRV_{Mem} (Equation 9). LRV_{MBR} represented overall process removal by comparing influent and permeate microorganism densities. LRV_{Bio} compares influent and mixed liquor densities and is representative of removal due to biopredation only. LRV_{Mem} considers mixed liquor and permeate densities and is representative of removal of microorganisms, either attached to suspended solids or freely suspended in the activated sludge, by the membrane and fouling layer.

$$LRV_{MBR} = \log_{10}\left|\frac{C_{In}}{C_{Perm}}\right| \quad \text{Equation 7}$$

$$LRV_{Bio} = \log_{10}\left|\frac{C_{In}}{C_{ML}}\right| \quad \text{Equation 8}$$

$$LRV_{Mem} = \log_{10}\left|\frac{C_{ML}}{C_{Perm}}\right| \quad \text{Equation 9}$$

where C_{In}, C_{ML} and C_{Perm} were the microorganism densities in the influent, activated sludge and permeate, respectively.

Lognormal probability density functions (PDFs) were fit to cumulative microorganism densities, before and after membrane change, with goodness of fit analysed by Root-Mean Squared Error. Monte Carlo simulation allowed the use of Equations 7, 8 or 9 considering microorganism PDFs, resulting in a PDF representing LRV_{MBR}, LRV_{Bio} and LRV_{Mem}. Monte Carlo simulation and PDF fitting were performed with @Risk software (Palisade Corporation, version 6.0) and Latin Hypercube sampling (using 10,000 iterations). Previous studies have used similar approaches in order to address limitations due to concentrations below permeate LOD and to account for performance variability (Olivieri *et al.* 1999, Khan *et al.* 2010, van den Akker *et al.* 2014). Lognormal distributions were previously shown as adequate for modeling parameters in treated and untreated wastewater (Oliveira *et al.* 2012). LRV PDFs were compared for each indicator before and after membrane replacement. In addition, LRV_{Mem} for the period before replacement was compared to clean water challenge test results on an aged membrane extracted from the plant.

9.3 Results

9.3.1 Operating parameters

Operating parameters for sampling periods before, immediately after and 6 months after replacement were summarised in Table 27.

Permeability for aged membranes was between 0.1 – 0.5 L/m^2/hr/kPa indicating significant fouling. As a result of fouling flux was limited likely due to TMP (43 – 50 kPa) exceeding the net positive suction of the permeate pumps. Permeability increased to 0.9 – 1.5 and 1.6 – 2.0 L/m^2/hr/kPa in the first month and 6 months after membrane replacement, respectively. As a result fluxes of 11 – 24 L/m^2/hr could be achieved. The significant increase in flux resulted in a sudden decrease in HRT, from 11 – 28 hr before to 4 – 11 hr after replacement.

Table 27 – Operating parameters for sampling periods at North Head MBR

Parameter	Sample Period (Number of Sample Days)								
	Before Replacement (22)			Immediately After Replacement (21)			6 Months After Replacement (16)		
	Min	IQR[a]	Max	Min	IQR[a]	Max	Min	IQR[a]	Max
Flux (L/m^2/hr)	5.2	8.3 – 15.5	21.6	16.6	22.8 – 23.7	23.8	10.6	21.8 – 23.7	23.8
TMP (kPa)	43.6	47.5 – 48.9	49.8	11.2	18.0 – 21.1	26.0	6.1	13.1 – 14.9	15.1
Permeability (L/m^2/hr/kPa)	0.11	0.17 – 0.33	0.50	0.92	1.12 – 1.32	1.48	1.56	1.58 – 1.69	1.96
pH	4.6	4.9 – 5.7	6.5	6.1	6.2 – 6.4	6.5	6.5	6.7 – 6.9	7.0
Temperature (°C)	18.4	19.7 – 21.0	21.8	21.2	22.3 – 23.8	24.4	21.4	21.7 – 22.4	22.8
HRT (hr)[b]	17	18 - 22	28	4	5 - 5	11	4	5 - 5	10
SRT (d)[c]	12	12 - 12	12	19	19 - 22	22	11	13 - 13	13
MLSS (g/L)	1.6	1.9 – 2.7	3.5	2.8	3.2 – 6.7	7.0	4.0	4.6 – 5.3	5.8
MLVSS (g/L)	0.7	1.5 – 2.1	2.8	2.4	2.8 – 5.5	5.7	3.1	3.8 – 4.0	4.3

[a] Interquartile range
[b] Calculated based on previous days total flow
[c] Calculated based on monthly total

SRT was approximately 12 days before replacement and 6 months after replacement. Following replacement sludge wastage was slowed in order to increase suspended solids concentration. Before replacement MLSS was 1.6 – 2.8 g/L, Immediately after replacement MLSS increased from 2.8 to 7 g/L. 6 months later, upon return to a 12 d SRT, MLSS had normalised to 4 - 5.8 g/L.

pH, ranging from 4.6 – 6.5, was lower than typical for an MBR before replacement . Following membrane replacement and suspended solids growth pH normalised from 6.1 – 7.0.

9.3.2 Microorganism removal

9.3.2.1 Clostridium perfringens

CP was detected only twice at a density of 2 CFU/100mL in the 22 permeate samples analysed before membrane replacement. Similarly, for the 37 samples taken immediately and 6 months after replacement CP was detected on 2 occasions at the LOD of 1 CFU/100 mL. Due to the low rate of detection of CP in the permeate, no LRV_{MBR} or LRV_{Mem} PDF was possible via Monte Carlo simulation. By dividing the influent density PDFs by the detected concentration a highly conservative 5th percentile LRV_{MBR} of > 4.6 and > 5.0 could be calculated for before and after replacement, respectively.

LRV_{Bio} did appear to shift to lower values, with a reduction in median removal from -1.3 to -1.5 from before to after replacement (Figure 13). CP has previously been reported to accumulate within the bioreactor, resulting in negative LRV_{Bio}, due to its ability to resist harsh environments (Marti *et al.* 2011, van den Akker *et al.* 2014).

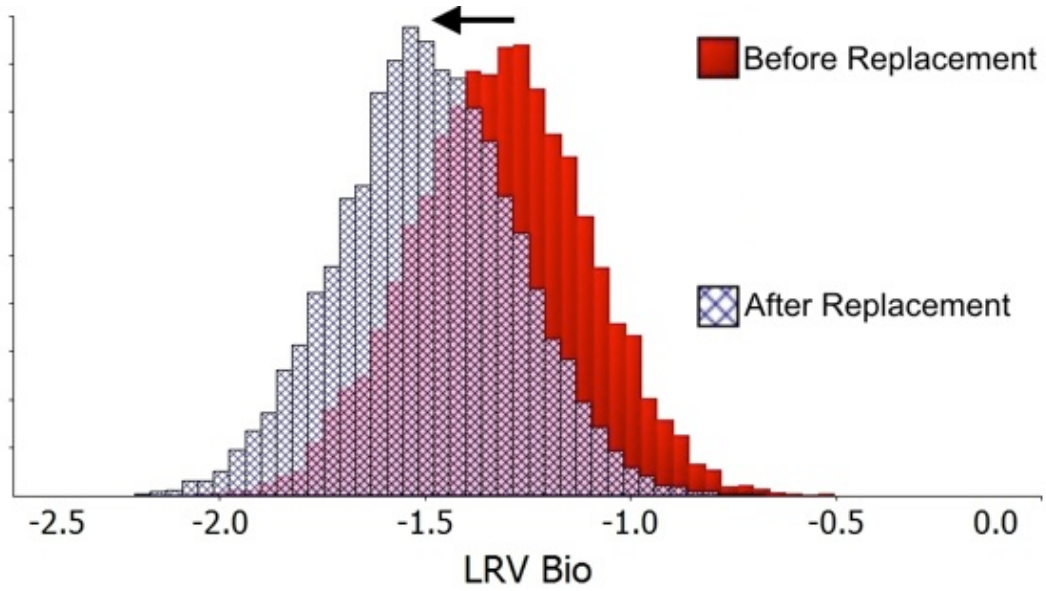

Figure 13 - Change in LRV_{Bio} for CP following membrane replacement.

9.3.2.2 E. coli

Similar with CP, EC LRV_{Bio} reduced by 0.5 LRV following membrane replacement. LRV_{Mem} increased significantly by 1.9 LRV resulting in a net increase of LRV_{MBR} by 1.3 (Figure 14).

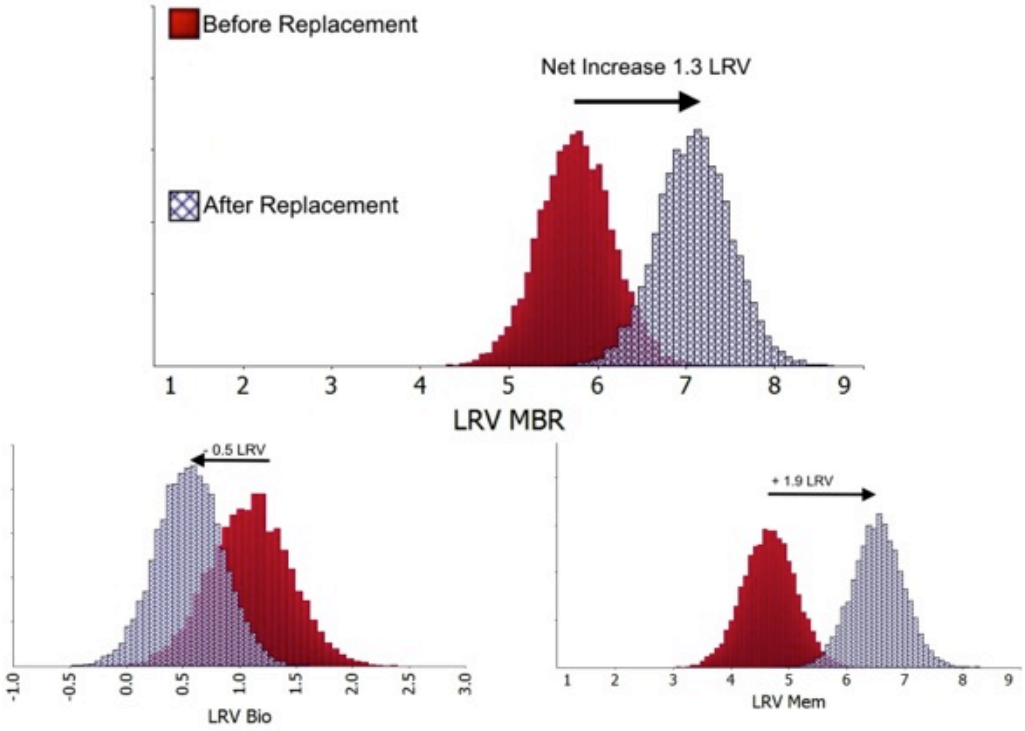

Figure 14 – EC LRV change after membrane replacement.

9.3.2.3 Somatic coliphage

SC LRV_{Bio} reduced by 0.6 LRV following membrane replacement, similar with EC and CP. LRV_{Mem} showed a slight reduction by 0.3 LRV in contrast to EC and CP. Prior to membrane replacement SC LRV_{MBR} 5^{th} percentile and median values were 3.6 and 4.4, respectively. Following membrane replacement, SC LRV_{MBR} 5^{th} percentile and median values were 2.8 and 3.5. Overall LRV appeared to reduce significantly by 0.9 (Figure 15).

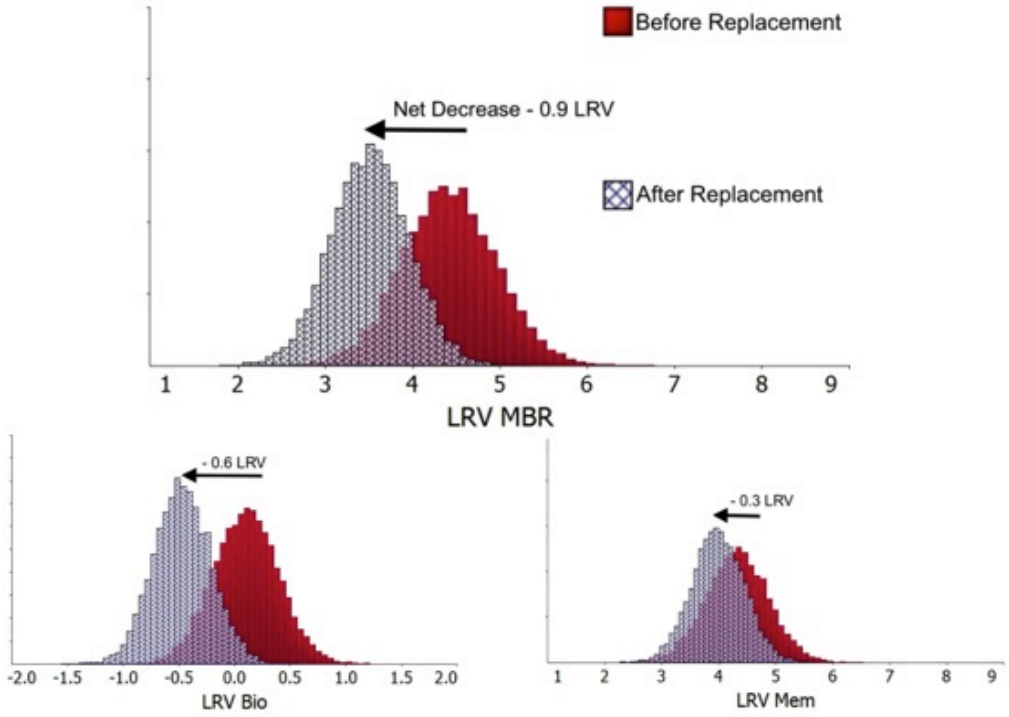

Figure 15 – SC LRV change following membrane replacement.

9.3.2.4 FRNA bacteriophage

A change in LRV_{Bio} could not be confirmed following membrane replacement as the standard deviation between distributions increased from 0.4 to 0.8. Median LRV_{Bio} shift was < 0.05. LRV_{MBR} Median and 5th percentile values reduced from 5.1 and 3.7 before and 4.5 to 3.4 after replacement, respectively. A majority of the overall LRV reduction was due to a 0.7 decrease in LRV_{Mem} following membrane replacement, similar to the behaviour of SC (Figure 16).

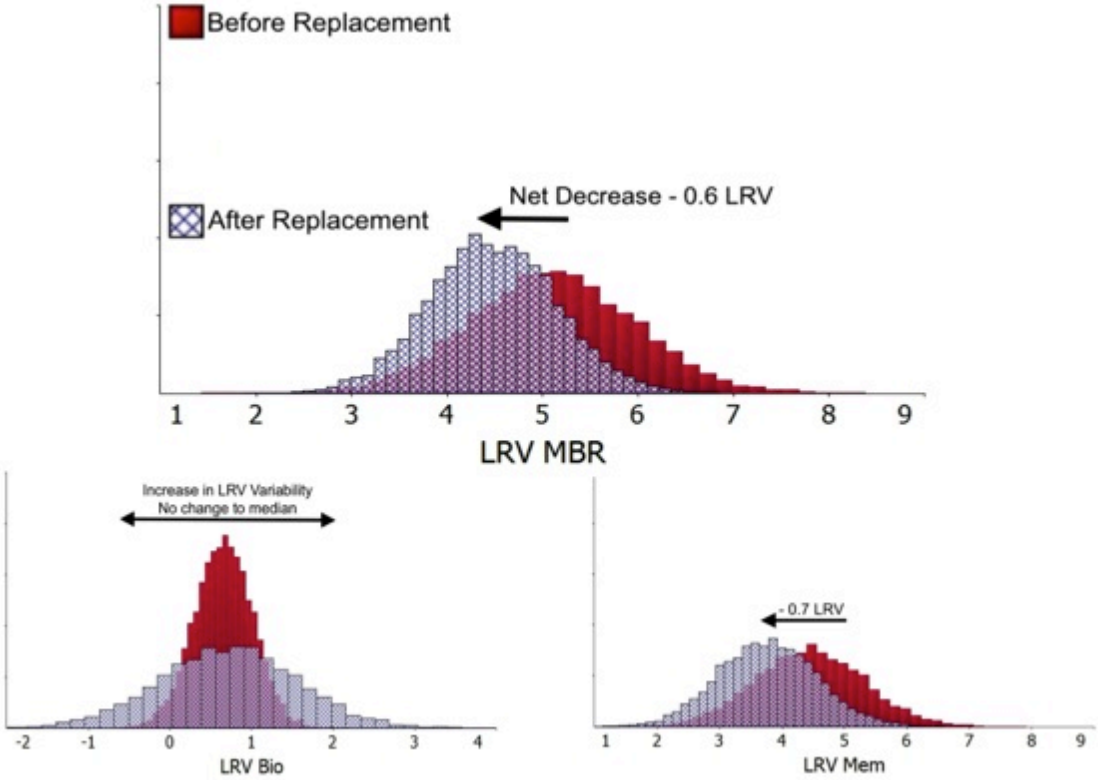

Figure 16 – FRNA LRV change following membrane replacement.

9.3.3 Challenge testing of the used membrane

In order to gain further insight into intrinsic membrane properties a single module was isolated and autopsied after removal for replacement. The module was also visually inspected and pressure decay tested. Visual inspection revealed at least 5 broken fibres. The fibres appeared to be broken close to the suction end of the membrane but were contained within accumulated rag like material. Pressure decay testing resulted in total pressure loss within 10 seconds, if averaged over 2 minutes pressure decay was > 14 and > 49 kPa/min for test pressures of 30 and 100 kPa respectively. A majority of leaks appeared to be confined to the broken fibres, no micro bubbling was observed.

Challenge testing was conducted in RO water spiked with laboratory cultured MS2 bacteriophage to determine standard virus removal and sewage from the site in order to determine clean water removal of indigenous indicators. Relative error between samples was less than 10% for all microorganisms tested. Permeability was between 1.0 and 1.5 $L/m^2/hr/kPa$ for all clean water tests. Clean water testing was conducted at 10 (CW LRV_{10}) and 30 $L/m^2/hr$ (CW LRV_{30}). For comparison clean water LRVs were compared to the 5th percentile and median in-situ measurements of LRV_{Mem} (Figure 17).

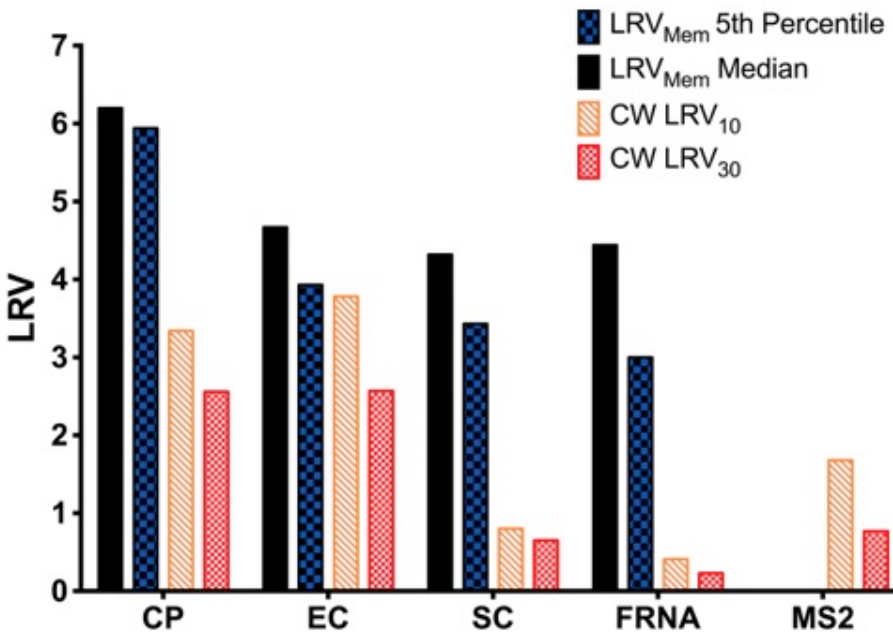

Figure 17 - Comparison between clean water test results and in-situ membrane rejection.

LRV_{Mem} for CP was > 5.9, clean water LRV was 3.3 and 2.6 at 10 and 30 L/m^2/hr, respectively. EC LRV_{Mem} median and 5th percentile were 4.7 and 3.9, compared to clean water rejections of 3.8 and 2.6 at 10 and 30 L/m^2/hr. LRV for SC was 0.8 and 0.7 in clean water but exhibited high median and 5th percentile LRVs in-situe of 4.3 and 3.4. Simillary, FRNA LRV in clean water decreased from 0.4 to 0.2 with increase in flux, whereas in-situ had median and 5th percentile LRV_{Mem} of 4.4 and 3.0. MS2 bacteriophage standard (ATCC #15597-B1) had a higher clean water rejection than indigenous FRNA of 1.7 and 0.8 at 10 and 30 L/m^2/hr respectively. For all indicators a reduction between 70% and 50% was observed with clean water LRV with an increase in flux from 10 to 30 L/m^2/hr. Clean water removal of virus indicators (SC and FRNA) was significantly lower than in-situ measurements. CP also exhibited lower clean water removal compared to in-situ results. EC removal at the 5th percentile corresponded closely with clean water results at 10 L/m^2/hr.

9.4 Discussion

After operating for 5 years, the log removal values of FRNA, SC, EC were analysed and reported (Pettigrew *et al.* 2010). LRV_{Bio} and LRV_{MBR} were extracted and alterations were made to permeate concentrations to ensure consistency with the LOD used in this study, effectively censoring some of the older data. Results were compared with LRV PDFs calculated from this study (Table 28).

Table 28 – Comparison of available data at 5 years.

Indicator	5 years[a]			10 years			After replacement		
	min	median	max	5th	median	95th	5th	median	95th
FRNA LRV_{MBR}	>3.8	>4.5	>4.9	3.7	5.1	6.4	3.4	4.5	5.6
FRNA LRV_{Bio}	-0.5	0.0	0.6	0.1	0.7	1.3	-0.7	0.7	2.1
SC LRV_{MBR}	3.7	4.6	>4.7	3.6	4.4	5.3	2.8	3.5	4.2
SC LRV_{Bio}	-1.0	-0.7	-0.5	-0.4	0.1	0.6	-0.9	-0.5	-0.1
EC LRV_{MBR}	5.4	5.7	6.7	5.1	5.8	6.5	6.4	7.1	7.8
EC LRV_{Bio}	0.0	0.6	0.7	0.5	1.1	1.7	0.1	0.6	1.0

[a] data taken from (Pettigrew *et al.* 2010)

LRV_{Bio} determined in this study appeared improved for FRNA, SC and EC when compared to results at 5 years. Upon change over of membranes, LRV_{Bio} for SC and EC more closely resembled the 5-year data. By comparison of the 5 and 10 year LRVMBR, no significant performance decline could be concluded, with median values and 5th percentiles in agreement to within 0.5 log units. Membrane replacement appeared to increase EC LRV_{MBR} by 1 log but FRNA and SC removal appeared to be lower at the 5th percentile.

The removal due to biological predation (LRV_{Bio}) cannot be mechanistically linked to the replacement of membranes. The change in membranes did result in significantly increased water productivity and as a result HRT was reduced from 17 – 28 to 4 – 11 hr. Although no corresponding operational data is available from the 5 year study of North Head, the design HRT is 5.5 hr. Assuming that hydraulic performances had not declined already at 5 years it is likely that the HRT at 5 years was similar to the value when the membranes were changed over. The results from this study, would suggest some relationship with improved LRV_{Bio} at longer HRTs.

Pressure decay testing and visual inspection indicated a significant damage rate on the membrane tested ex-situ. The rate of membrane damage was in the same order of magnitude with previous estimates of 1 fibre breakage per year (Gijsbertsen-Abrahamse *et al.* 2006). EC (0.5 – 1 μm) and CP (1 μm) are typically larger than the membrane pore size (0.04 μm). The significantly enhanced LRV_{Mem} (+1.9 log units) of EC can be explained by the restoration of size exclusion ability of the newly replaced membranes. Clean water testing LRVs for the aged membrane EC and CP at 10 $L/m^2/hr$ were 80 and 50% of the corresponding LRV_{Mem} medians. SC and FRNA clean water LRVs were 20 and 10% of in-situ LRV_{Mem}, respectively. Similar values (0.4 ± 0.1) were reported for MS2 bacteriophage by a microfiltration membrane in clean water (Shang *et al.* 2005). The presence of suspended solids in an MBR would appear to improve removal by 20 – 50% for larger microorganisms and up to 90% for smaller bacteriophages, even in the presence of significant membrane damage. The significant increase in membrane permeability upon replacement correlated with a decrease in LRV_{Mem} of smaller virus indicators SC and FRNA. Previous studies indicated a reduction in permeability from by 50% corresponded to somatic coliphage removal increases of 1 log (Farahbakhsh *et al.* 2004), consistent with results presented in this study.

9.5 Conclusions

Over 10 years of MBR operation, membranes become damaged and heavily fouled. The amount of damage would appear to result in loss of size exclusion ability of larger microorganisms; however, in the presence of suspended solids, total loss of size exclusion did not exceed 1.5 LRV or approximately 20% when compared with new membrane rejection. The presence of fouling significantly improves virus rejection, greater than that possible with virgin membranes, even when membrane integrity is severely compromised. Bio predation limits accumulation of microorganisms rejected by the membrane. The effectiveness of bio-predation is microorganism specific, but in all cases in this study, biopredation was reduced at shorter HRTs. In situations where high log removal value is sought, careful control of operational conditions and maintenance and monitoring of membrane integrity is recommended.

10 Correlation of Turbidity with MBR LRV

10.1 Introduction

Experimental work is underway with the aim of investigating methods to correlate turbidity with LRV. Preliminary aims and findings are included, subject to change, in the following section.

The chosen online monitoring technique must be correlated to LRV. The limitation of the chosen online monitoring technique resolution should yield a maximum demonstrated LRV. The VDoH guidelines have proposed a method for correlation of turbidity with LRV. Previous validation reports have set a critical control limits (CCL) for turbidity at 0.2 – 0.5 NTU, based on research from membrane suppliers. Generally, when CCLs are exceeded, a timeframe specified to reduce chance of false positive is considered before corrective actions are implemented. Control strategies have included bypass to head of works or waste, or plant shutdown. The critical control limit and corrective actions must be documented as part of the recycled water quality management plan.

An example approach to correlate turbidity, measured in MBR permeate, with LRV is presented below. A minimum of 6 paired samples over different permeate turbidities should be taken of influent and permeate with LRVs calculated with Equation 7. Before attempting to correlate turbidity and LRV, ensure that turbidity meters are cleaned, calibrated and installed as per manufacturer instructions. In order to generate higher turbidities, two approaches are plausible:

- **Approach 1:** Use a dosing pump to bypass mixed liquor into the permeate line at increasing dosages while noting the bypass ratio. Begin at the lowest ratio and finish at the highest ratio. Correlate the bypass flow with an expected membrane damage rate, based on a flow dilution model. Express LRV and turbidity results as illustrated in Figure 18.
- **Approach 2:** Sequentially damage membranes by systematically cutting fibres or slicing sheets in order to allow bypass of MLSS particles into the permeate. Record turbidity and damage rate. It may be necessary to backflush membranes with air or liquid to avoid turbidity recovery due to plugging of membrane defects with activated sludge flocs.

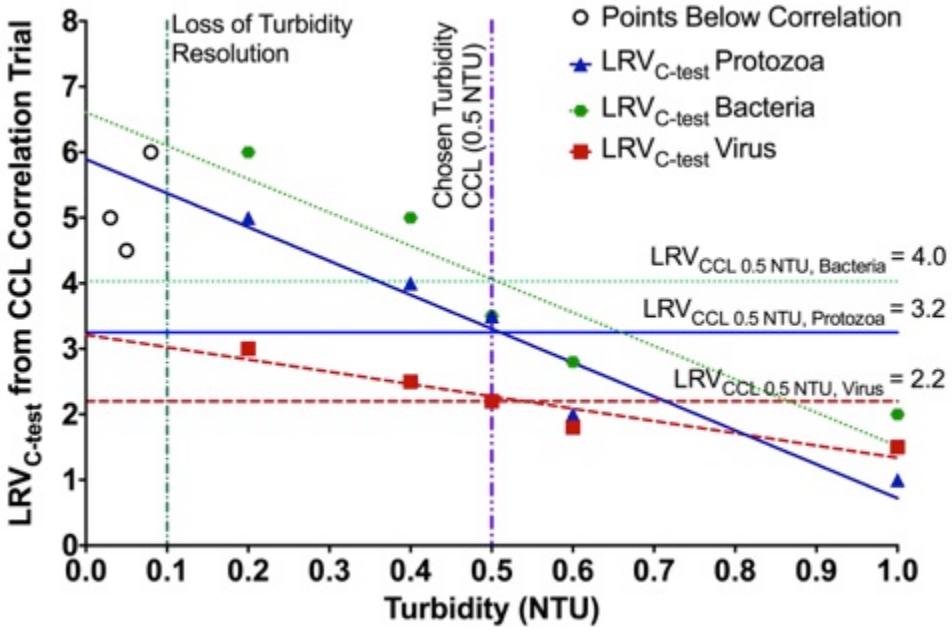

Figure 18 - Illustration of CCL determination for turbidity. Not real data.

After following either Approach 1 or 2, data should be available to allow creation of a plot similar to Figure 18. A CCL for turbidity can then be chosen and a corresponding LRV_{CCL} selected at the point where the $LRV_{C\text{-}test}$ correlation meets the chosen turbidity. It is likely that significant loss of resolution will occur at low turbidities. Sampling should only take place where there is a certain measurable change. Before designing a sampling program, it may be worth assessing historical turbidity data to ascertain the normal baseline. The CCL must be chosen within the range of correlated values and greater than the value where loss of resolution occurs. The sampling program should be conducted under the same conservative conditions identified in the validation methodology. Turbidity correlation should be performed at the lowest MLSS concentration in the operating envelope.

10.2 Preliminary sensitivity analysis modelling of turbidity and LRV

The activated sludge in MBR nominally contains 4000 – 20000 mg/L of suspended solids (SS), that are removed by an intact membrane. Transfer of these solids to the permeate, detectable by turbidity, would indicate a failure in the process. A 15L solution was circulated through laser turbidity meters. Activated sludge from a full scale MBR, was spiked into the tank at increasing doses. The bulk concentration of *E. coli* and SS was correlated with turbidity.

Theoretical concentrations, based on dilution, and measured values of SS and *E. coli* were correlated with turbidity. Theoretical concentrations were higher than measured, resulting in more conservative correlations. Both *E. coli* and SS turbidity correlations were linear up to the maximum instrument range of 5000 mNTU (Figure 19).

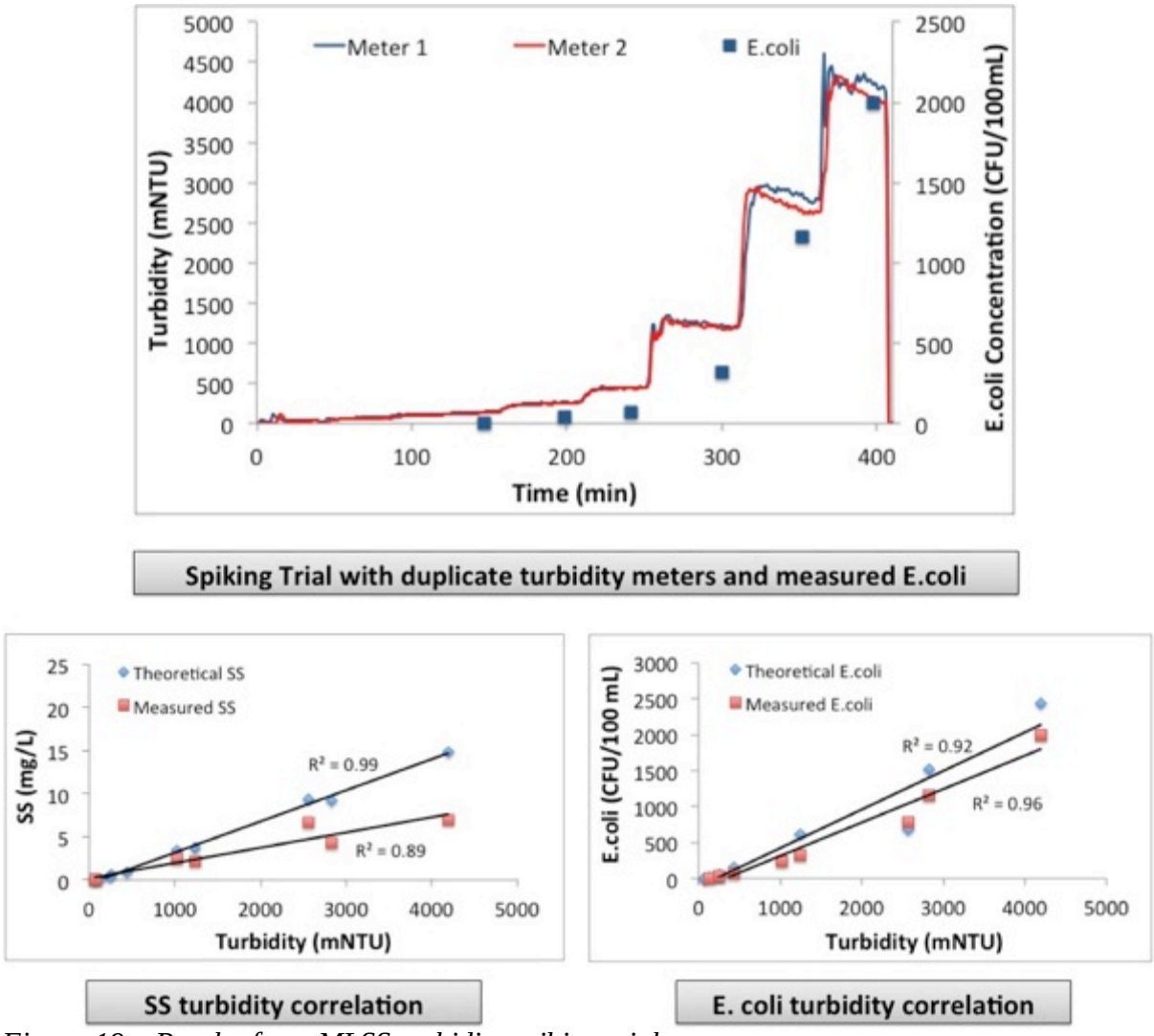

Figure 19 – Results from MLSS turbidity spiking trials.

10.2.1 Modelling procedures and results

E. coli feed densities (n = 30 samples) from a full scale MBR were fit to a lognormal probability distribution function [CFeed(lognormal)]. The correlation between *E. coli* and turbidity was transformed into a linear function, to calculate *E. coli* permeate density based on turbidity [CPermeate(Turbidity)]. For each permeate turbidity a normal distribution of LRV resulted, that encompassed possible feed water variability (Figure 20).

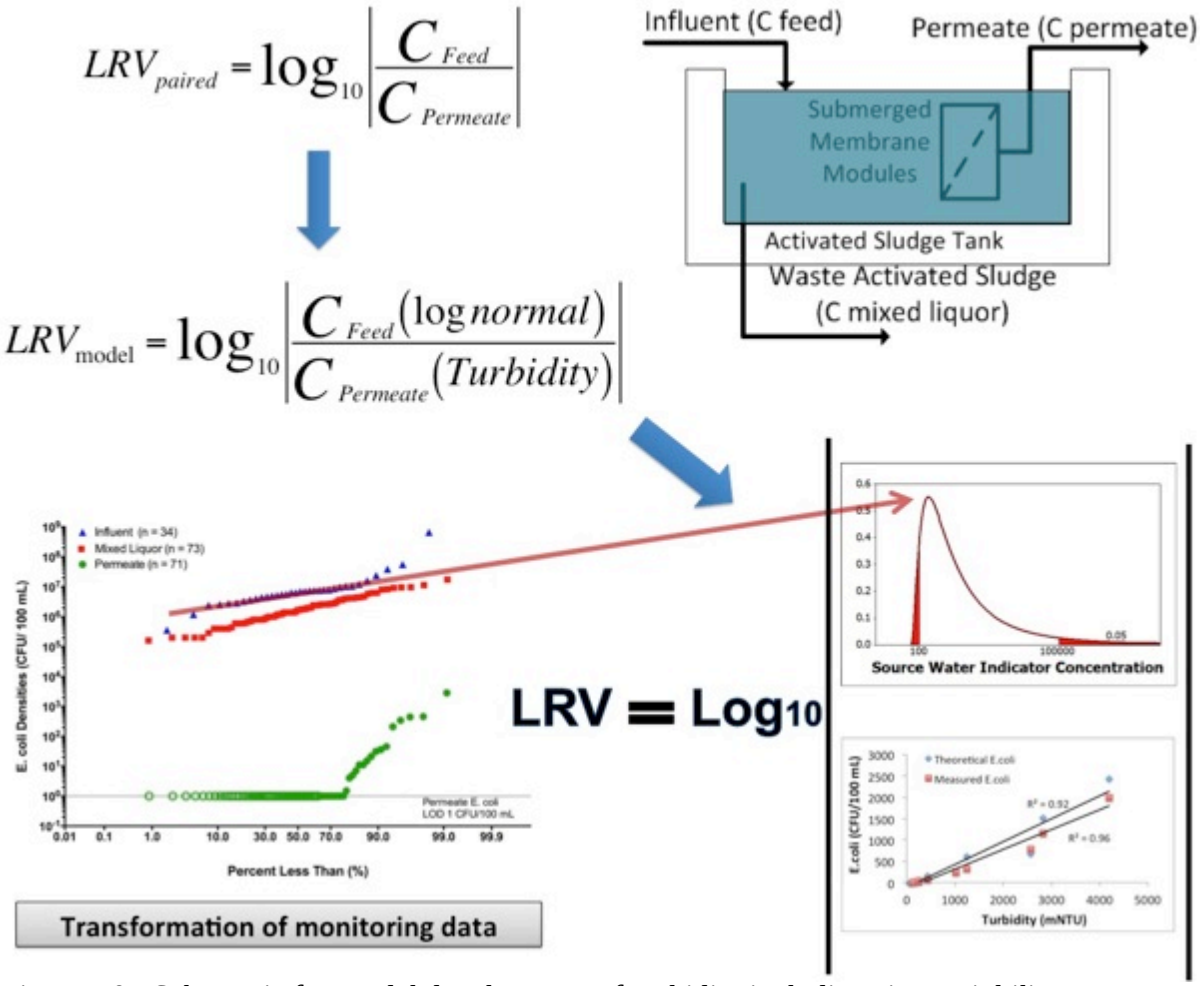

Figure 20 - Schematic for model development of turbidity including site variability.

The turbidity model accounted for feed water variability, with error bars representing 5th and 95th percentile LRV for a given turbidity. The model was conservative, predicting LRVs below the paired validation set, for turbidity > 250 mNTU (Figure 21).

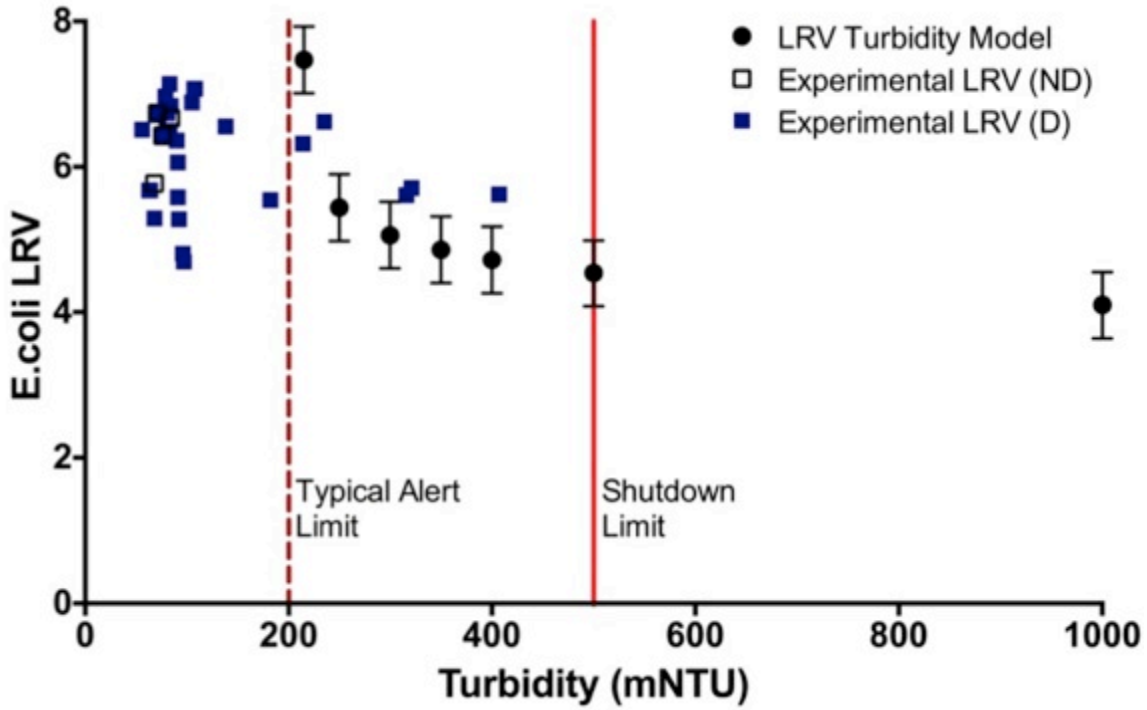

Figure 21 - Turbidity model and paired E. coli LRV data from a full scale MBR.

10.3 Interim conclusions and continuing work on turbidity

The model predicted appropriately conservative LRVs at higher turbidities (> 250 mNTU). A bacteria LRV of 3 – 4 appears justified via turbidity as a critical control point.

Further studies will be conducted with intentionally damaged membranes to validate the correlation at higher turbidities. The effect of SS particle size on turbidity response will be assessed. Classification of false positives/negatives below 200 mNTU is necessary. Assessment of more full scale sites will be conducted to determine whether the model is site specific or could be global. Assessment of protozoan and virus indicators will be similarly modelled. Assessment of other rapid testing methods is also underway.

11 Assessment of Hazardous Events on MBR: Impact on Bulk Water Quality Parameters

11.1 Introduction

In many countries, meeting stringent water quality discharge requirements for sensitive streams or the implementation of water recycling treatment processes first requires validation that the process is capable of achieving water quality requirements. In order to fully validate the performance of membrane bioreactor (MBR) systems, frequently used for these applications, it is necessary to investigate their performance under various operational conditions. In the field of risk assessment, a deviation from normal operational conditions is commonly termed a 'hazardous event' (van den Akker *et al.* 2014) and investigating impacts of hazardous events on process performance is an important aspect of treatment process validation. Hazardous event is a key aspect of the risk assessment philosophy adopted by the World Health Organisation (WHO) for the application of Water Safety Plans (World Health Organisation 2009) and the Guidelines for Drinking Water Quality (World Health Organisation 2011). The formalised consideration of hazardous events has been applied for a range of risk assessment and risk management applications including managing waterborne diseases (Mouchtouri *et al.* 2012) and managing chemical accidents (Jang *et al.* 2011). Hazardous events that may affect the operation of wastewater treatment systems can include sudden changes in source water composition, extreme weather events, human error and mechanical malfunctions (Ren 2004, Trinh *et al.* 2014). There have been a number of studies previously reporting the use of chemical shock experiments to assess the performance of conventional activated sludge (CAS) wastewater treatment processes (Kincannon *et al.* 1968, Saleh *et al.* 1978, Li *et al.* 1999, Ng *et al.* 2005, Henriques *et al.* 2007). However, there are currently no studies on the contribution of such hazardous events to the risk of treatment failure or underperformance in MBRs. This paper presents the first part of a series of complementary studies addressing the impacts of hazardous events including salinity shock, 2,4-dinitrophenol (DNP) shock, ammonia shock, organic carbon shock, feed starvation, loss of power supply, loss of aeration, complete wash out of biomass, defective fibres and physical membrane damage. The present work focuses on the impact of those hazardous events on the removals of key operational parameters and bulk water quality using a laboratory-scale MBR system.

Salinity shock, ammonia shock and organic carbon shock scenarios were selected for this study as they are commonly reported to exhibit short peak loads in full-scale wastewater treatment plants (WWTPs) (Kincannon *et al.* 1968, Selna *et al.* 1979, Hart *et al.* 2003). DNP shock was selected as a representative peak load caused by electron inhibitors as it is a well-known inhibitor of efficient energy production in cells with mitochondria (Mayhew *et al.* 1998, Low *et al.* 1999). It uncouples oxidative phosphorylation by carrying protons across the mitochondrial membrane, leading to a rapid consumption of energy without generation of adenosine triphosphate (ATP) and, at high concentrations, can disrupt a variety of important bacterial metabolic processes (Brummett *et al.* 1977, Decker *et al.* 1977, Nicholas *et al.* 1978, Bakker *et al.* 1984, Henriques *et al.* 2005). The other hazardous events for MBRs selected for this investigation were identified through an expert workshop at the beginning of the study. These hazardous event studies can assist with validation of MBR processes and facilitate better environmental and human health risk management for MBR systems.

11.2 Materials and methods

11.2.1 Chemical substances

NaCl, NH_4HCO_3, DNP, glucose and glutamic acid (analytical grade) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

11.2.2 Experimental MBRs

A laboratory-scale MBR test system was comprised of four identical experimental MBRs (30 L each), fed from a single continuously-mixed influent tank (Figure 22). Each MBR was designed to operate

with a solids retention time (SRT) of 30 days, a hydraulic retention time (HRT) of 1 day resulting in an average organic loading rate of 0.32 kg COD.m^{-3}.d^{-1}, and a flux of 10 L.m^{-2}.h^{-1}. The HRT of 1 day was selected as full-scale package MBR plants in previous baseline studies usually operate at this long HRT and relatively low organic loading rate (Le-Minh *et al.* 2010, Trinh *et al.* 2012, Trinh *et al.* 2012, van den Akker *et al.* 2014).The test systems were located at a local WWTP to facilitate testing with the use of primary treated effluent filling a common influent tank (200 L) daily. This filling process involved screening through a 1 mm fine screen mesh. The screened contents of the influent tank are subsequently referred to as the 'influent' to the MBR systems. Mixing in the influent tank was maintained with gentle stirring from a mechanical mixer. Characteristics of the influent are presented in Table 29. Initially, the four bioreactor tanks were seeded with biomass from an existing pilot-scale MBR operating at the same WWTP. This system had mixed liquor suspended solid (MLSS) concentrations ranging from 5 to 12 g.L^{-1} and had been treating the same primary effluent for approximately one year. As such, the biomass was well acclimatised to the feed.

Table 29 - Characteristics of the influent (n = 40).

Quality parameters	**Unit**	**Influent (mean ± stdev)**
pH		7.4 ± 0.4
Chemical oxygen demand (COD)	mg.L^{-1}	321 ± 89
Dissolved organic carbon (DOC)	mg.L^{-1}	61 ± 32
Total nitrogen (TN)	mg.L^{-1}	87 ± 46

The influent was fed from the influent tank to the MBRs by gravity. A cistern valve was used to control the influent flow for each reactor. The aerobic chamber (20 L) of each MBR was intermittently aerated with 15 minutes on/off cycles to stimulate nitrification (aerobic) and denitrification (anaerobic) microbial processes. A further mechanical mixer in each aerobic chamber was used to maintain a well-mixed solution. The membrane chambers (10 L each) were aerated continuously to assist biofouling control. Peristaltic pumps (Masterflex, model no. 07551-00) were used for permeation. To facilitate membrane relaxation, the permeate pump was turned off manually for 10 minutes every day. Transmembrane pressure (TMP) was monitored online by pressure transducers (Tempress Controls, Gosford, NSW) while the data were logged on a computer.

The membranes used in this study were polyvinylidene-difluoride (PVDF) membranes that were manufactured by Evoqua Water Technologies (South Windsor, NSW, Australia). Each membrane had an internal diameter of 800 µm, external diameter of 1300 µm and a pore size of 0.04 µm. Each MBR contained four membrane modules, each consisting of 30 fibres with an average fibre length of 27 cm resulting in a total permeation area per reactor of 0.13 m^2.

The system design included the ability to backwash membrane modules when the TMP was observed to exceed a specified maximum value (50 kPa). However, the TMP did not exceed this value at any time during the study. The combination of low flux and continuous membrane aeration was shown to be effective for minimising biofouling and therefore membrane backwashing was not required.

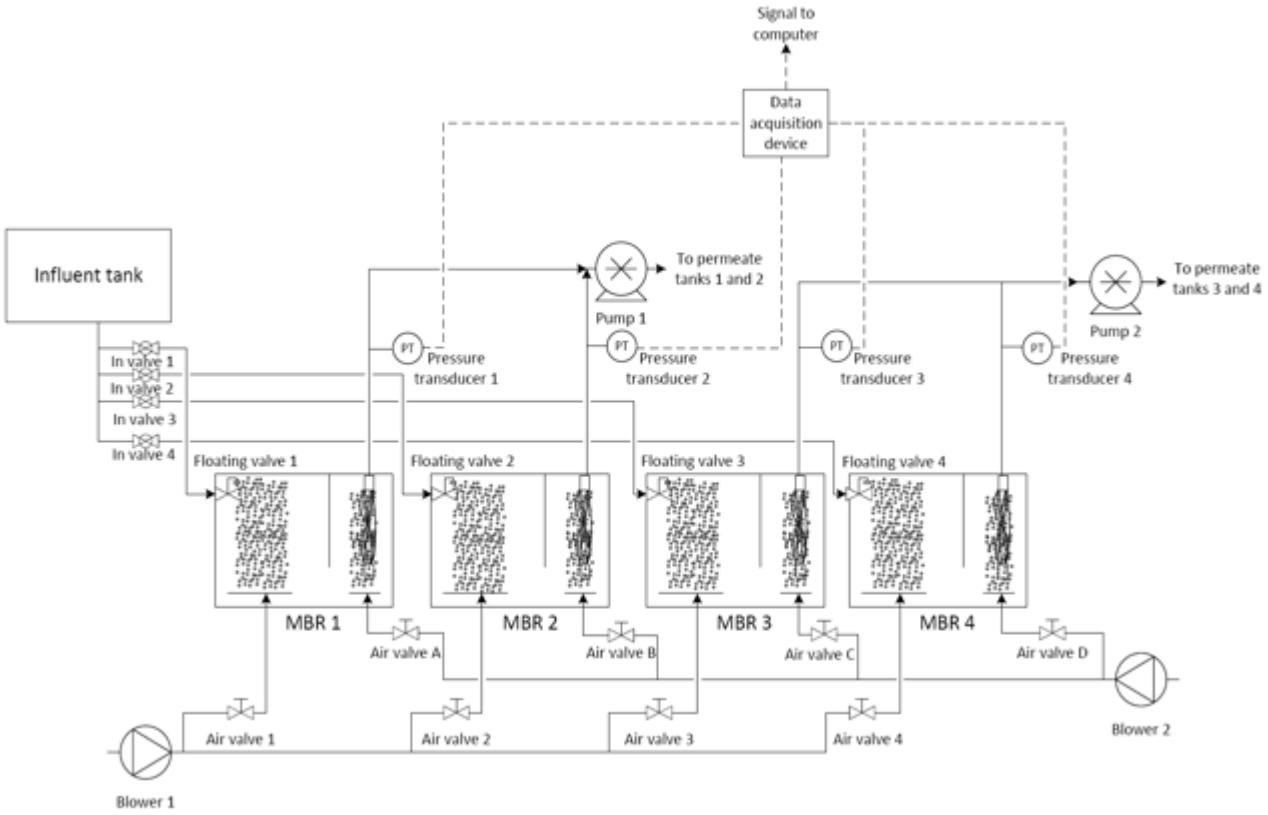

Figure 22 - Schematic illustration of the MBR system.

11.2.3 Control experiments

Prior to commencement of the hazardous event simulation experiments, all MBRs were operated under the same conditions for one week to assess the reproducibility of performance between the four parallel systems. In addition, during the hazardous event simulation experiments, one of the four MBRs was operated under steady-state conditions as an experimental control while the other three MBRs were subjected to hazardous events.

11.2.4 Shock load simulation experiments

Based on previous studies on shock loads in CAS systems (Kincannon *et al.* 1968, Saleh *et al.* 1978, Li *et al.* 1999, Ng *et al.* 2005, Henriques *et al.* 2007) and experience with full-scale MBR operation (Severn 2003), a dose of 200 $mg.L^{-1}$ DNP was selected for the DNP shock experiment, a dose of 20 $g.L^{-1}$ NaCl was selected for the salinity shock experiment, a dose of 700 $mg.L^{-1}$ ammonia was selected for the ammonia shock experiment and a dose of 5 $g.L^{-1}$ of COD was selected for the organic carbon shock experiment. These elevated concentrations were selected to represent potential worst case scenarios with the anticipation that there would be a noticeable impact on MBR performance.

The shock load simulation experiments were scheduled into two separate trials. The first trial included salinity shock, ammonia shock, DNP shock experiments and a control. The second trial included organic carbon shock experiment and a control. The MBRs were operated at steady-state for at least one day before being subjected to the shock simulations. DNP, NaCl and NH_4HCO_3 were each introduced as a single shock-dose to the bioreactors of each MBR of the system. The organic carbon shock was introduced in the form of a mixture of glucose and glutamic acid (1:1) to represent a range of assimilable organic compounds. The mixture was also introduced as a single dose to the bioreactor tank of one of the MBR.

Influent was sampled daily after refilling and mixing of the influent tank. Permeate and mixed liquor were sampled prior to introducing the shocks and at 1 hour, 24 hours, 48 hours (and 72 hours when possible) after introducing the shocks. Influent and permeate samples were analysed for pH, COD,

and DOC. Mixed liquor samples were analysed for MLSS, mixed liquor volatile suspended solid (MLVSS), and capillary suction time (CST). The pH value was determined using a 5-Star portable pH meter from Thermo Scientific Orion. COD, MLSS and MLVSS measurements were carried out according to Standard Methods for Examination of Water and Wastewater (American Public Health Association *et al.* 2013). CST was measured by a Triton Type 319 multipurpose CST. TMP was also continuously monitored throughout these experiments and data were used to estimate and contrast fouling rates (Le-Clech *et al.* 2006).

11.2.5 Operational hazardous events

The 'operational hazardous events' simulated included (1) starvation; (2) loss of power; (3) loss of aeration; (4) complete wash out of biomass; and (5) defective in membrane fibres. For all experiments, influent was sampled daily after filling the influent tank and TMP was continuously monitored.

Starvation conditions were simulated by stopping the feed to the MBR for 6 days. During the starvation period, the permeate pump remained on and the permeate was recycled to the MBR to maintain a constant water volume in the system. During the starvation period, permeate samples were not taken from the control or the starvation MBRs. After 6 days under starvation conditions, the MBR feed was restarted. Permeate was sampled from both the control and the starvation MBRs, at 1 hour, 24 hours, 48 hours and 72 hours after re-establishing the feed to the systems. Mixed liquor samples from both the control and the starvation MBR reactors were taken daily during the experiment including the starvation period when plant access was available.

The loss of power shock conditions were created by terminating the power supply to the system for a duration of 2 hours. This power loss resulted in loss of feed flow, aeration of the bioreactor, membrane air scour, as well as permeation. Permeate and mixed liquor samples from the control and the shock reactor were taken immediately prior to terminating the power supply. During the period without power, permeate samples were not taken from the experimental or control MBRs. After 2 hours under the loss of power conditions, power supply was resumed and the MBR was operated as normal. Permeates and mixed liquors were sampled from both the control and the shock MBR at 1 hour, 24 hours, 48 hours and 72 hours after power was returned to the system.

The loss of aeration condition was established by pausing air supply to the MBR for 24 hours. Permeate and mixed liquor samples from the control and the shock reactor were taken before stopping aeration as well as after stopping aeration at 1 hour, 24 hours and 48 hours.

For the complete loss of biomass scenario, the biomass was removed from the MBR so that influent water was filtered directly. For the defective fibres experiment, one out of 4 membrane modules of the MBR was replaced with a module made of industrial used membranes. Scanning Electron Microscopy images showed defects in the industrial used fibres ranging from 5 – 50 µm whereas the new membranes used throughout the experiments had pore sizes of 0.04 µm. The pressure decay rate of the 4 membrane modules in the defective fibres experiment was 16.5 $KPa.min^{-1}$ while the pressure decay rate of the new membrane modules was < 1 $KPa.min^{-1}$. Permeate and mixed liquor samples from the control and the shock reactors were taken after the events at 1 hour, 24 hours, 48 hours and 72 hours.

11.2.6 Physical membrane damage experiment

Preliminary laboratory tests were conducted to determine the extent and appropriate method for membrane damage. This involved cutting submerged hollow fibre membranes into two parts at various depths and monitoring the impact on permeate turbidity. During these tests, it was observed that if the membrane was cut at insufficient depth (less than 3 cm), the top of the capillary fibre would float on the surface and water would not be drawn through it. Cutting a single membrane (at lower depth) had negligible impact on permeate turbidity, however, cutting two (or more) membranes led to a drastic increase in permeate turbidity (>300 NTU). The different impacts between cutting the first and second fibre implied that there was a significant element of 'chance' regarding whether a specific fibre breakage would be blocked (leading to negligible turbidity rise) or remain open (leading to drastic turbidity rise). Subsequently, the experimental protocol for this hazardous event simulation involved sequential cutting of membranes until a major turbidity breach was observed.

The final physical membrane damage simulation was undertaken by initially cutting one membrane fibre (resulting in damage ratio of 0.8%) by a sharp knife at a depth of 10 cm. After cutting the membrane fibre, effluent was sampled directly from the permeate pump tube every minute for 10 minutes, and immediately analysed for turbidity (COD was later analysed in the laboratory). Based on turbidity results, a second fibre of the same module was cut (resulting in damage ratio of 1.6%) at a similar position (10 cm depth) and permeate was continuously sampled from the tube of the permeate pump every minute for 20 minutes, then at 1 hour, 2 hours, 3 hours, 24 hours and 48 hours for subsequent turbidity and COD measurements.

11.3 Results and Discussion

11.3.1 Control experiments

Removal of COD and DOC above 90% was achieved by all four MBRs during the initial phase to test reproducibility. These results were consistent with previous MBR studies (DeCarolis *et al.* 2007). The results including permeate pH, COD removal, DOC removal, MLSS, MLVSS concentrations and CST were reproducible between the four MBRs with a maximum standard deviation of 3%. In order to characterise operational parameters and removal performances under normal conditions, data from the reproducibility experiments, from sampling immediately before hazardous events, and from the control MBR (resulting in a minimum of 60 data points for each parameter) were used as a point of reference to determine performance impacts during the various shock scenarios. The mean and standard deviations of each performance parameter as a function of shock scenario are represented in Figure 23, Figure 24 and Figure 25. The overall standard deviations account for all variations, both within each MBR as well as between the MBRs, under normal operational conditions over the entire hazardous event experimental period. The hazardous event was considered as having no significant impact if the results were inside the control range (mean ± 1 standard deviation). In contrast, if the results were outside the control range, the hazardous event was deemed as having a significant impact on the investigated parameter.

11.3.2 Shock load simulation experiments

The impacts of salinity, DNP, ammonia and organic carbon shock conditions on change of COD removal, DOC removal, pH, MLVSS concentrations and CST over time (48 hours) are presented in Figure 23.

11.3.2.1 COD and DOC

COD removal efficiency had reduced considerably after one hour of introducing the salinity shock (Figure 23A), which is consistent with a number of previous studies on MBR (Reid *et al*. 2006, Yogalakshmi *et al*. 2010) and CAS reactor performance (Ludzack *et al*. 1965, Kincannon *et al*. 1966, Kincannon *et al*. 1968, Ng *et al*. 2005). These studies reported that COD removal decreased drastically when influent NaCl concentrations reached 20 g.L-1 due to saline conditions producing a higher osmotic pressure on bacterial cells, therefore inhibiting bacterial growth and floc formation (Dan *et al*. 2003). The salinity shock load of approximately 20 g.L-1 has been observed as a consequence of 70% seawater ingress to a leaking sewer, leading to a full-scale MBR plant in the UK (Severn 2003). A decrease in COD removal efficiency was linearly correlated with increasing influent NaCl concentrations between 20 and 60 g.L-1 (Ng *et al*. 2005). COD removal in the salinity shock experiment recovered to the control range within 24 hours after introducing the shock. This agreed with permeate conductivity data which indicated that the majority of NaCl had been washed out of the MBR by 24 hours. A slight reduction in DOC removal was observed to have occurred by 1 hour after shock, which had further reduced by 24 hours and then recovered back to within the control range by 48 hours (Figure 23B).

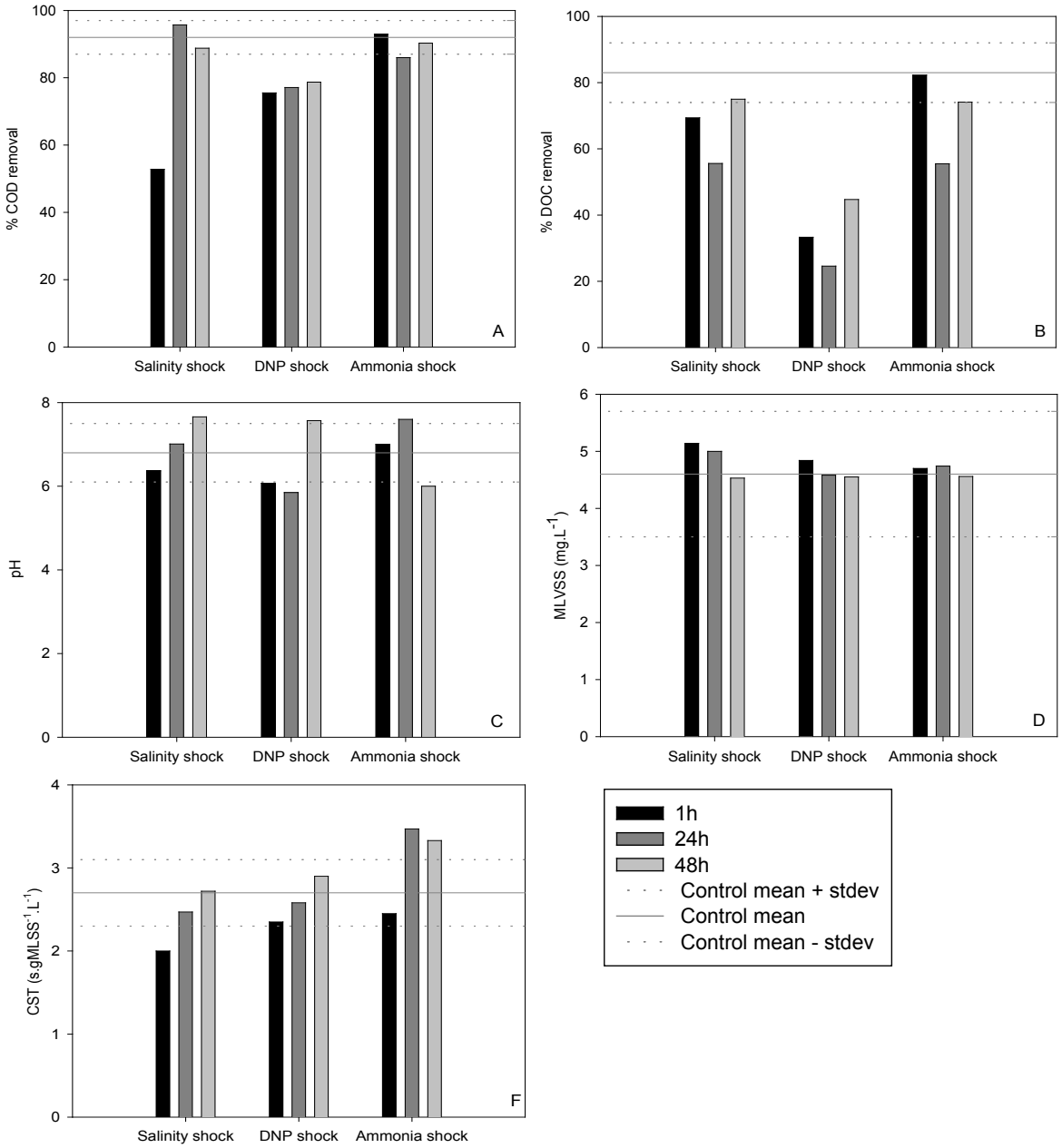

Figure 23 - Change of (A) COD removal; (B) DOC removal; (C) pH; (D) MLVSS; and (F) CST as a function of salinity, DNP and ammonia shock experiments after introducing the shocks at 1 hour, 24 hours and 48 hours.

One hour after introducing the DNP shock, COD removal had also reduced, but to a lesser extent than in the case of salinity shock (Figure 23A). A wide variety of DNP concentrations have been reported for the inhibition of COD removal efficiency for AS systems. One study using a batch CAS reactor fed with synthetic wastewater found that at 20 mg.L-1 DNP, COD removal reduced from 90% to 53% (Chen *et al*. 2006). In contrast, a study using a sequencing batch reactor fed with municipal wastewater reported no effect on COD removal efficiency at DNP concentration up to 107 mg.L-1 (Henriques *et al*. 2007). It has been hypothesised that some variation may be explained by variable endogenous concentrations of DNP (or other chemicals with similar properties) in municipal wastewaters, and hence, the presence of variable populations of DNP-degrading bacteria in WWTPs (Jo *et al*. 1998). The experiment reported here was conducted in an MBR fed with real municipal wastewater and real biomass originating from a pilot-scale MBR. Hence, the presence of DNP-degrading bacteria is likely. However, the shock dose of 200 mg.L-1 was selected such that it would exceed the likely tolerance of the biomass in the MBR, leading to observable inhibition of

biodegradation processes and a reduction in COD removal efficiency. 48 hours after DNP shock, a partial recovery in COD removal was observed, though it was still 8% lower than the control range. Samples from 1 hour and 24 hours after DNP shock both showed DOC removal to have been reduced to a greater extent than COD removal. After 48 hours, DOC removal had partially recovered, however, it was still 29% lower than the control range (Figure 23B).

For the ammonia shock experiment, there was only a slight reduction observed in COD removal by 24 hours, whereas DOC removal had considerably decreased (Figures 23A and 23B). The COD and DOC removals then recovered back within the control range by 48 hours after shock.

For organic carbon shock load, previous studies have shown that activated sludge processes (with MLSS concentration of 2 $g.L^{-1}$) were quickly recovered after a shock load of 3 $g.L^{-1}$ COD (Saleh *et al.* 1978). Since MBRs have higher MLSS concentration and are expected to be more resilient to COD shock load, a shock dose of 5 $g.L^{-1}$ was selected in this study. As some industrial wastewaters have very high COD concentrations, for example, starch processing wastewater has COD up to 13 $g.L^{-1}$ (Huynh *et al.* 2004), cheese processing wastewater has COD up to 15 $g.L^{-1}$ (Torrijos *et al.* 2004), and pharmaceutical processing wastewater has COD up to 360 $g.L^{-1}$ (Mart´ınez *et al.* 2003), sporadic discharge of such industrial wastewaters to MBR plants due to unexpected events (or illegal dumping) could conceivably result in a 5 $g.L^{-1}$ COD shock load concentration. In the organic carbon shock experiment, as COD was introduced directly into the MBR, COD and DOC permeate concentrations are presented for the control and the organic carbon shock experiments (Figure 24).

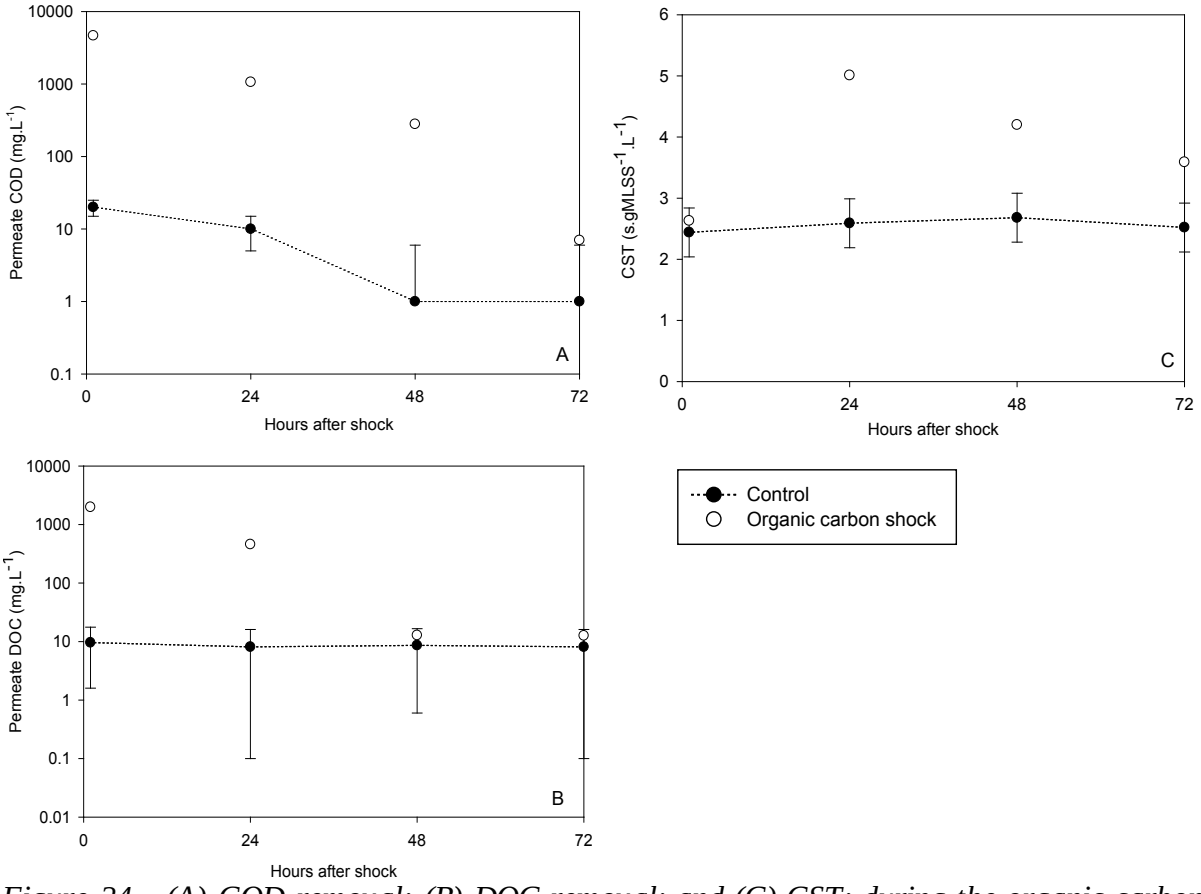

Figure 24 - (A) COD removal; (B) DOC removal; and (C) CST; during the organic carbon shock experiment.

The results obtained for the organic carbon shock experiment (Figure 24A) show that the COD of the permeate increased sharply from 29 $mg.L^{-1}$ to 4650 $mg.L^{-1}$ within 1 hour. If the COD from the mixed liquor is used to calculate COD removal efficiencies, then the COD removal decreased significantly from 89 % to 12 % after 1 hour. It is therefore evident that COD removal had, in fact, been impacted and that the increase in COD concentration in the permeate was not simply proportional to the

increased COD in the mixed liquor. The COD concentrations of the permeate in the shocked reactor were reduced to 1066 $mg.L^{-1}$ after 24 hours, 280 $mg.L^{-1}$ after 48 hours and further decreased to 7 $mg.L^{-1}$ after 72 hours (Figure 24A). This result is consistent with previous studies of CAS systems, which report that the influent shock concentrations around 3 $g.L^{-1}$ COD caused significant increases in effluent COD concentrations (Saleh *et al.* 1978, Manickam *et al.* 1985). Four to six days after a 3 $g.L^{-1}$ COD shock was applied, the COD in a CAS effluent returned to a low level (Saleh *et al.* 1978, Manickam *et al.* 1985). In contrast, another study reported that organic carbon shock loads with influent COD concentration from 5 to 16 $g.L^{-1}$ COD provided no significant impact on the permeate COD of an immersed MBR system (Al-Malack 2007). This inconsistency may be due to the synthetic wastewater used, which may contain easily biodegradable organic carbon compounds. In addition, the MBR (Al-Malack 2007) was operated at much higher MLSS concentration (15 $g.L^{-1}$) than the MLSS concentration in the present study (around 5 $g.L^{-1}$). This higher MLSS may provide some resilience against sudden COD increases in the influent. The impacts of organic carbon shock on DOC removal confirm the trend observed for COD removal (Figure 24B).

These results suggest that changes in COD and DOC removal efficiencies are effective indicators for monitoring impacts of hazardous events such as salinity, DNP and organic carbon shocks.

11.3.2.2 pH

The pH of the MBR permeate in the organic carbon shock experiment decreased by 3.6 units within the first hour. This was likely due to the large amount of glutamic acid added in the reactor, which exceeded the metabolic rate of the existing microorganisms. Thus, the organic acid accumulated in the reactor leading to a decrease in pH of the mixed liquor and MBR permeate. The pH value slightly rose after 24 hours and fully recovered to the control range (6.8 ± 0.7) within 48 hours indicating that the microorganisms had metabolised the accumulated glutamic acid in the reactor. The pH of the permeate was unaffected by other shock load conditions (Figure 23C).

11.3.2.3 MLSS and MLVSS

MLVSS concentrations were unaffected by any of the investigated shock load conditions (Figure 23D). The MLSS results also followed a similar trend to the MLVSS results. All observed variations of MLSS and MLVSS results were within the control range (5.8 ± 2.3 for MLSS and 4.6 ± 1.1 for MLVSS).

11.3.2.4 CST and fouling rate

The CST of the mixed liquor from the ammonia shock experiment had significantly increased after 24 hours (Figure 23F), implying that the filterability of the mixed liquor from the ammonia shock experiment had reduced. This is in agreement with previous CAS studies which revealed that activated-sludge settling and dewatering properties can deteriorate at ammonia concentrations higher than 20 $mg.L^{-1}$ (Novak 2001). Monovalent cations such as ammonium exchange with divalent cations in the floc, weakening the binding biopolymers and resulting in weaker and less-dense flocs (Higgins *et al.* 1997, Novak 2001). The CST of mixed liquor from the ammonia shock experiment had recovered slightly after 48 hours, but was still 6% higher than the control range.

The organic carbon shock experiment also showed significantly increased CST after 24 hours (Figure 24C). In previous CAS studies, organic shock loads have been found to cause a decrease in floc-size, changes in dominant microorganism types, and changes in the biochemical composition of the sludge (Saleh *et al.* 1978, Manickam *et al.* 1985, Seetha *et al.* 2010). These changes may be the cause of a reduction in the filterability of the mixed liquor after being subjected to the organic carbon shock.

Changes in fouling rates were not easily detected for the four shock loading experiments due to the large variations in TMP values during the control experiments. However, fouling rates were found to be slightly greater within the first 24 hours after introducing the feed shocks into the MBRs (data not shown). The results also revealed that the initial increase in fouling rate during the ammonia shock experiment was easily reversed through the relaxation period (10 minutes) applied after 24 hours. The impacts of the other shocks on fouling rates were still present after 72 hours indicating the irreversible nature of the fouling layer formed during shock conditions.

Previous studies have reported that a natural response of bacteria upon exposure to a toxic shock was an increase in the release of soluble microbial products (SMP) and extracellular polymeric

substances (EPS) into the mixed liquor (Love *et al.* 2002, Aquino *et al.* 2004, Chen *et al.* 2006, Reid *et al.* 2006). This is usually the cause of increases in CST and fouling property in MBRs under toxic shock conditions (Reid *et al.* 2006, Judd *et al.* 2011). In addition to the impacts on key operational and bulk water quality parameters, the organic carbon shock was observed to create foaming in the reactor and cause overflow of biomass out of the reactor within the first 48 hours after shock.

11.3.3 Operational hazardous events

The impacts of starvation, loss of power, loss of aeration, complete wash out of biomass and defective fibre conditions on COD removal, DOC removal, pH, MLVSS and CST are illustrated in Figure 25.

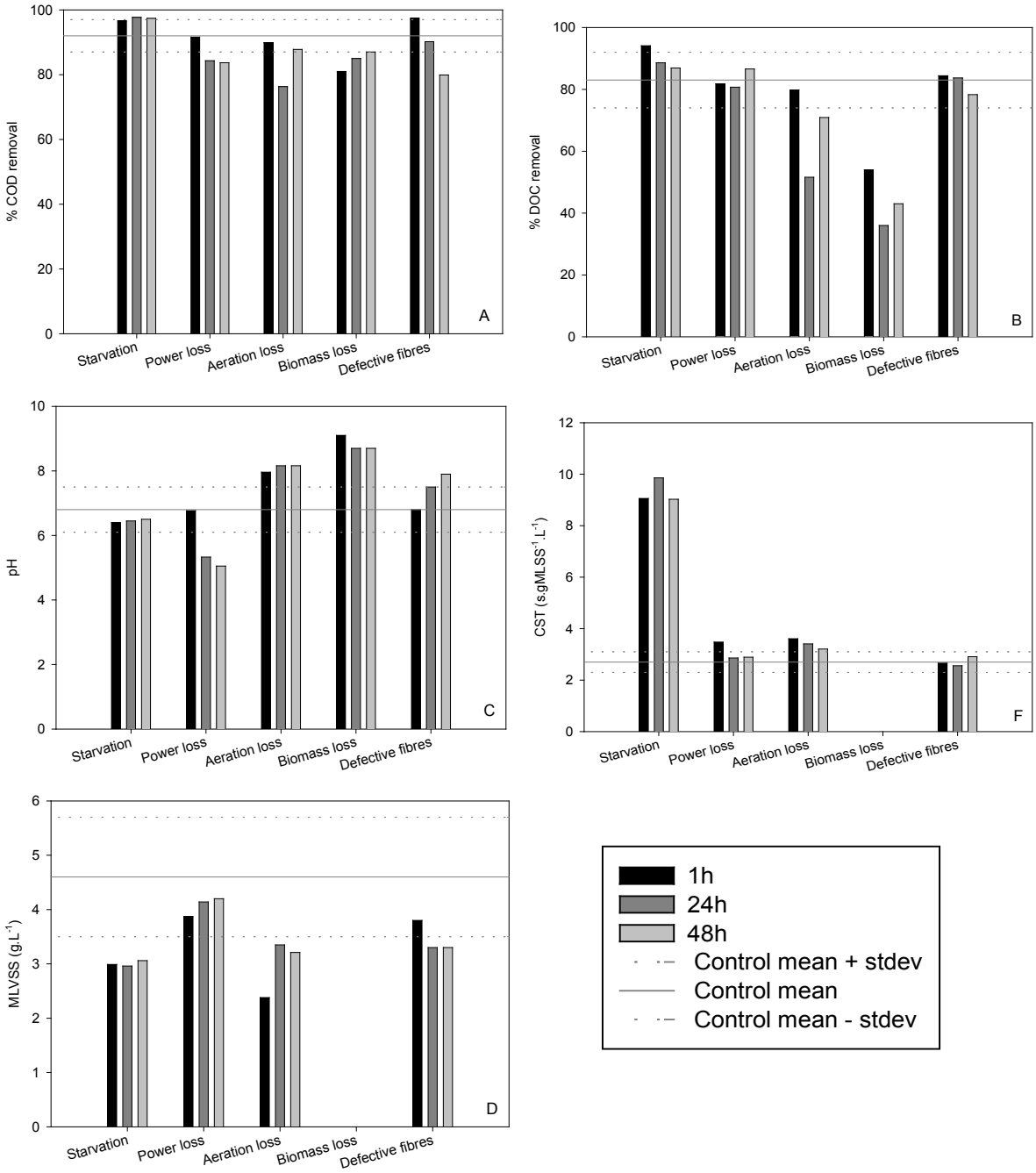

Figure 25 - A) COD removal; (B) DOC removal; (C) pH; (D) MLVSS; and (F) CST during starvation, power loss, aeration loss, complete biomass loss (no biomass) and defective fibres experiments at 1 hour, 24 hours, and 48 hours.

11.3.3.1 COD and DOC

Results presented in Figure 25A and B suggest that after re-feeding the starved MBR, COD and DOC removal efficiencies in the reactor immediately increased to 97% and 94%, respectively. This suggests a rapid microbial utilisation of the available carbon sources after the long starvation period (Li *et al.* 2006). The high COD and DOC removals were stable and remained within the control range until the end of the experiment. A previous study found that the DOC and nitrogen removal by laboratory-scale MBRs was not affected under a feed starvation shock of 2 days (Le-Minh 2011). In this study, the starvation period was extended to 6 days and the COD removal efficiency reached the same value as the control experiment immediately after re-feeding. This suggests that MBR systems can withstand the tested starvation conditions and can recover quickly after re-feeding back to pre-shock steady-state conditions. Another laboratory-scale MBR study for a starvation period of 5 days reported that, 3 days after re-feeding, the COD removal efficiency reached 90% and fully recovered back to steady state conditions after 6 days of normal operation (Yogalakshmi *et al.* 2007). The MBR in this previous study had MLSS concentration of 15 $g.L^{-1}$, which is considered to be relatively high when compared to typical MLSS concentrations in MBRs in practice, and three times higher than MLSS concentration in the current study (5 $g.L^{-1}$). The difference in MLSS concentrations may be the reason for the longer recovery time, as the concentrations of the dead biomass may be larger. The concentration of organic matter released from dead cells to the liquid medium thus may be higher (Coello Oviedo *et al.* 2003) and such systems require longer recovery times.

Results presented in Figure 25A illustrate that loss of aeration led to an 11% decrease in COD removal after 24 hours, though COD removal recovered to within the control range by 48 hours. DOC removal followed a similar trend as COD results, as after 24 hours performance was reduced 22% in comparison to the lower limit of the control range (Figure 25B). The DOC removal had essentially recovered by 48 hours, but was still 3% smaller than the control range.

COD removal in the complete loss of biomass reactor was slightly lower (6%) than the control range, 1 hour after the event. It then returned to within the control range by 48 hours after the event (Figure 25A). However, DOC removal in the reactor was considerably lower than the control range 1 hour after the event, and was still 31% lower than the control range 48 hours after the event (Figure 25B). COD removal in the loss of power and defective fibres experiments was slightly lower than the control range 24 hours and 48 hours after the events (Figure 25A) while DOC removal performance in these experiment was within the control range (Figure 25B).

11.3.3.2 pH

The pH of the permeate for the complete loss of biomass reactor was approximately 1 unit higher than both the pH of the influent and permeate of the control, indicating that some biological or chemical transformation processes were occurring in the reactor. The higher pH in the permeate may be due to changes in equilibrium within the shock reactor due to the absence of biomass. Permeate pH in the loss of aeration reactor was also slightly higher than the control range, while the permeate pH of the loss of power reactor was slightly lower than the control range (Figure 25C).

11.3.3.3 MLSS and MLVSS

After starvation, MLVSS concentration (Figure 25D) was 17% lower than the control range and remained at this level until the end of the experiment. The MLSS data showed a similar trend as the MLVSS data. This result is consistent with previous studies of CAS systems, which reported that biomass concentrations in CAS decreased sharply during the first 4 days of the starvation period (Urbain *et al.* 1993, Coello Oviedo *et al.* 2003). A previous study on MBR also found biomass concentration reduced significantly after a starvation period of 5 days with it taking almost a month of continuous operation to regain the amount of biomass lost during feed starvation (Yogalakshmi *et al.* 2007). Feed starvation seemed to have a significant impact on MLSS and MLVSS concentrations, but the overall system performance remained relatively resilient to the starvation shock as it continued to achieve effective COD and DOC removals.

A 30% reduction in MLVSS concentration was observed 1 hour after the aeration lost which maybe due to insufficient mixing in the reactor after loss of aeration resulting in not representative samples. After the aeration was turned back on, the MLVSS concentration was just 4 to 8 % lower than the control range. The power loss duration over a 2 hour period caused no significant impacts on MLSS and MLVSS concentrations.

11.3.3.4 CST and fouling rate

The CST was observed to increase significantly during feed starvation (Figure 25F). In previous CAS studies, starvation conditions have been reported to cause a degradation of both proteins and polysaccharides in the sludge (Urbain *et al.* 1993). Starvation shocks also result in the disappearance of some of the typical microbial groups usually present in a CAS system and the appearance of other opportunistic microorganisms (Coello Oviedo *et al.* 2003). These may be the cause for the considerable increase in CST under starvation conditions. The loss of power caused an immediate increase in CST, but recovered back to within the control range at 24 hours. CST in the loss of aeration experiment was slightly higher than the control range during the experiment.

The fouling rate of the starvation reactor was about 60% higher than the control within 48 hours after feeding was stopped, though it returned to within the control range by 72 hours. During the aeration loss experiment, the fouling rate was about 7 times higher than the value obtained during the control experiment, but returned to the control range as soon as aeration was restarted. The fouling rate for the loss of biomass reactor was significantly higher than the control during the first few days of operation and continued to increase considerably until the last day of the experiment (up to about 40 times higher than the control range), indicating unsustainable operation. Finally, the defective fibres and loss of power events showed no impact on fouling rate.

The loss of power and loss of aeration events were also found to create significant biomass deposition blocking the air diffusion system. However, the problem was quickly recovered after the power and the aeration was reinitiated.

11.3.4 Physical membrane damage

The turbidity and COD of MBR permeate from the control and the physical membrane damage reactors, for the first 20 minutes after the fibres were cut are illustrated in Figure 26 and Figure 27. Turbidity of the MBR permeate was not affected after cutting the first fibre. The turbidity continued to closely match the values of the control (0.2 NTU) for 10 minutes, however, after cutting the second fibre, the turbidity in the MBR permeate immediately increased to 49 NTU by 1 minute and to 360 NTU by 3 minutes. It then decreased to 4 NTU by 7 minutes suggesting that biomass had clogged and sealed the breakage. The turbidity reduced to 0.3 NTU after 9 minutes and then increased to 1.1 and 1.2 NTU at 13 minutes and 14 minutes, respectively indicating a decrease in clogging. The turbidity was reduced back to 0.4 NTU after 15 minutes, gradually reduced to 0.2 after 18 minutes, and remained stable at this level until the end of the experiment (48 hours). The results confirm that turbidity is a good performance indicator for online monitoring, providing instant indication of physical membrane damage.

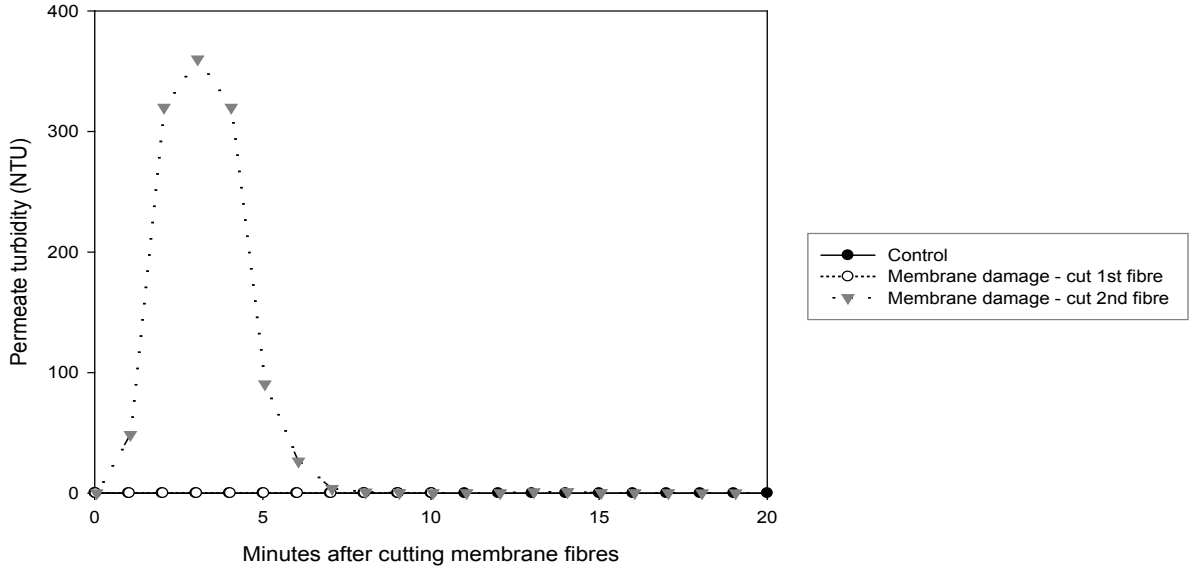

Figure 26 - Permeate turbidities of the control and the physical membrane damage experiments for the first 20 minutes after cutting fibres.

Permeate COD concentrations of the control and the physical membrane damage experiments are presented in Figure 27. After cutting the first fibre, the COD concentration in the MBR permeate increased from 24 to 48 mg.L^{-1} over 3 minutes, reduced to 33 mg.L^{-1} by 4 minutes, and remaining stable at this level. After cutting the second fibre, the COD concentration in the permeate immediately increased to 124 mg.L^{-1} after 1 minute, reducing to 51 mg.L^{-1} after 10 minutes indicating further leakage of dissolved organic matter through the membrane breakage. Such a membrane failure is expected to impair permeate water quality (Judd *et al.* 2011). The COD slowly reduced to 35 mg.L^{-1} after 60 minutes and remained stable at this level until the end of the experiment (48 hours). The results indicate that permeate COD concentration is also a potentially useful indicator for monitoring physical membrane damage conditions. However, COD is yet to be measured as an online analytical technique.

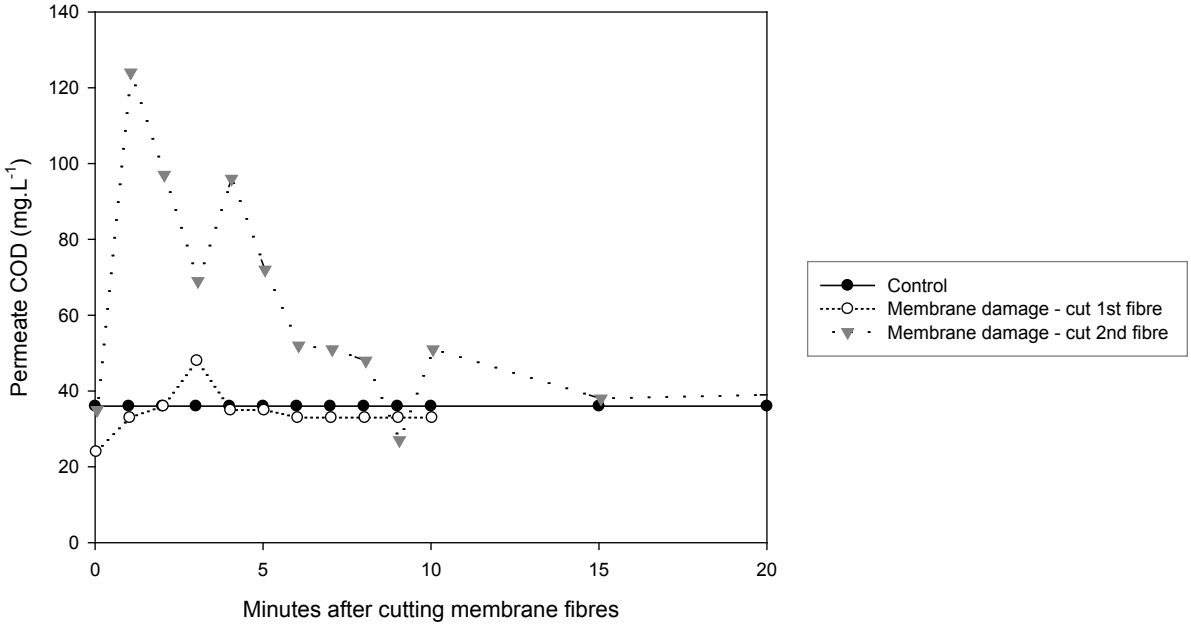

Figure 27 - Permeate COD concentrations of the control and the physical membrane damage experiments in the first 20 minutes after fibre cutting.

11.4 Conclusions

This is the first study that comprehensively investigated the impacts of a wide range of hazardous events on the operational parameters and key bulk water quality parameters of MBRs. The outcomes of this study will therefore facilitate greater understanding and validation of MBR processes. The main conclusions are:

- Significant reductions in COD and DOC removals were observed immediately after salinity shock, DNP shock and organic carbon shock, indicating that COD and DOC removals are effective parameters for monitoring the impacts of these hazardous events. Ammonia shock led to an immediate increase in fouling rate that was easily reversible through the relaxation period applied to the MBR 24 hours after shock, while increased fouling rates in other shock load reactors were still high 72 hours after shocks, indicating that the biomass and its impact on fouling did not recover.
- Starvation had a noticeable effect on MLVSS and MLSS concentrations, but nonetheless, the systems appeared to be resilient in terms of COD and DOC removal efficiencies. MLVSS and MLSS concentrations may be sensitive indicators of feed starvation. However, this indication may not necessarily translate into immediate performance problems.
- Turbidity and COD analyses in the physical membrane damage experiment revealed that any direct impacts were 'self-repaired' by the blocking of the breakage within approximately 15 minutes. The results confirmed that turbidity is a suitable performance indicator for online monitoring and able to quickly detect physical membrane damage. Permeate COD

concentration changes are also a potentially sensitive indicator for detecting physical membrane damage, but is limited by its offline nature.

- High removal rates of COD were maintained throughout the loss of biomass experiment. However, the fouling rate continued to increase considerably during the experiment, indicating unsustainable operation.

This study has identified which types of hazardous event have led to observable impacts in MBR treatment performance and permeate water quality. Future research could aim to better understand the mechanistic phenomena resulting from those hazardous events. For example, advanced microbial activity study is expected to identify specific changes in biomass characteristics during the shock loads.

12 Assessment of hazardous events on MBR: Impact on microorganism LRV

12.1 Introduction

Membrane bioreactors (MBR) are frequently used as a barrier in water recycling schemes, where biological nutrient removal is required and plant footprint is constrained (Lesjean *et al.* 2011). The primary hazard in water recycling are pathogenic microorganisms originating from sewage, due to the potential for acute health effects from exposure to low dosages (van den Akker *et al.* 2014). In water recycling applications, a thorough understanding of pathogen removal performance and variability for each treatment barrier is imperative. Any event compromising the pathogen removal efficiency must be detected and quantified to inform appropriate corrective action (Trinh *et al.* 2014). The mechanisms for pathogen removal in a MBR are size exclusion, entrainment within activated sludge flocs or membrane fouling layer and biological predation (Hai *et al.* 2014). Previously, theoretical simulations of hazardous events (Friedler *et al.* 2008) and shock loading of MBRs with domestic chemicals (Knops 2010) have been conducted, but without measurement of pathogen removal.

In this study, indicator organisms FRNA bacteriophage (FRNA), *Escherichia coli* (EC) and total coliforms (TC), and *Clostridium perfringens* (CP) were chosen to represent pathogenic viruses, bacteria and protozoa. Log removal values (LRV) were quantified during operation under normal, and hazardous event conditions. Direct measurement of pathogenic species in wastewater is often not feasible due to low and highly variable concentrations and complex analysis procedures (Antony *et al.* 2011). As a result indicator organisms are often chosen as surrogates for pathogens. A suitable indicator organism should be chosen such that it displays correlated or more conservative removal than the target pathogen (VDoH 2013). FRNA have been investigated in several previous studies of log removal in MBR (Severn 2003, Ottoson *et al.* 2006, Hirani *et al.* 2010, Pettigrew *et al.* 2010, Francy *et al.* 2012, Hirani *et al.* 2012, van den Akker *et al.* 2014). FRNA was selected as an indicator of virus removal performance due to its small size (0.025 µm) (Antony *et al.* 2011) and low iso-electric point (pH 3.9) (Michen *et al.* 2010). With a diameter of 0.025 µm, FRNA presented a substantial challenge to removal via size exclusion by the membrane (pore diameter generally larger than 0.04 µm) and was chosen to model similarly sized enteroviruses present in wastewater. The low iso-electric point (pH 3.9) relative to the typical operating pH of MBR (7-8) (Judd 2011) reduced the likelihood of adsorption of FRNA to the membrane, as above pH 3.9 the virus particle carries a net negative charge (Antony *et al.* 2011). Hence, FRNA was chosen as the virus indicator given, well-documented previous use and its conservative model properties. EC and TC were chosen to represent bacterial pathogens, due to their extensive historic use as fecal contamination indicators and as challenge organisms for membrane systems. CP was selected as a surrogate for protozoa. Due to CPs ability to form spores and resist hard environments, it has been used as a surrogate for cryptosporidium in disinfection studies (Venczel *et al.* 1997). Depending on the strain analysed, CP spore diameters range between 0.6 – 1 .0 µm (Orsburn *et al.* 2008). CPs smaller size, relative to other protozoa (5 – 10 µm) (Antony *et al.* 2011), further supports its use as a conservative indicator in membrane challenge testing. Additionally, CP has been used as a challenge organism to represent protozoan removal in previous studies on MBR (Ottoson *et al.* 2006, Marti *et al.* 2011, van den Akker *et al.* 2014).

As part of a larger investigation into operational resilience of MBRs, this paper is the third in a series that has previously assessed the impact of hazardous events on key bulk water quality parameters (Trinh *et al.*) and trace organic chemical removal. The aim of this study was to quantify the impact of hazardous events on removal of indicator organisms. Even under normal conditions, performances of wastewater treatment processes are inherently variable. Through the use of Monte Carlo simulation, hazardous events are evaluated with respect to process variability under normal conditions. Benchmarking against the magnitude of normal variability provided a realistic measure and ranking of hazardous event consequence. New knowledge has be provided as a result of this study that supports application of quantitative health risk management practices for MBRs in water recycling.

12.2 Experimental

12.2.1 Microbial Analysis

CP, EC, TC and FRNA were analysed according to previously published culture methods (van den Akker *et al*. 2014). Data was reported in colony forming units (CFU) for bacterial indicators and plaque forming units (PFU) for phage per 100 mL volume of sample.

Initial densities of indigenous FRNA were too low to provide meaningful results; removed to the limit of detection (LOD), within the activated sludge, before reaching the membrane. Spiking a lab culture, grown from FRNA indigenous to the waste water treatment plant (WWTP), into the feed tank, increased FRNA concentration during trials. The lab culture was first extracted from the top of positive plates using tryptone water (Oxoid). The extract was centrifuged and filtered through 0.45 µm syringe filters (Sartorius). The extract was then plated and incubated according to the double agar layer method used for analysis. A second, more concentrated, solution was then extracted from the incubated plates, centrifuged, and excess bacteria filtered out with 0.45 µm gridded filter membrane (Millipore). The re-incubation step was repeated until the resulting stock solution had a final concentration of approximately 10^{9} – 10^{11} PFU $(100\ mL)^{-1}$.

12.2.2 Operation and Sampling

Further detail on rationale for hazardous event selection, operation and sampling is provided elsewhere (Trinh *et al.*). Trials were conducted for 5 to 6 days at a time. At the trial beginning, 30 L lab scale MBRs were seeded with activated sludge from a larger pilot MBR, operated continuously at the WWTP. The solids retention time (SRT) of the larger pilot MBR was 30 days. The 30 L MBRs were operated at constant flux (10 $L.m^{-2}.h^{-1}$) and with a hydraulic retention time (HRT) of 24 hours. The activated sludge compartment was aerated intermittently (15 min on/off cycles) to promote nitrification and denitrification. Fouling was mitigated by constant aeration of the membrane compartment and relaxation for 10 minutes each day. Commercial hollow fibre membranes were sourced from Evoqua Water Technologies for construction into mini-modules. Four bespoke membrane modules were installed per reactor, having a total area of 0.13 m^2. The membranes were made of polyvinylidene difluoride (PVDF), with an outer diameter of 1300 µm and a nominal pore size of 0.04 µm. Four 30 L MBRs were operated in parallel and fed with sewage from a common feed tank (200 L). The feed tank was refilled each day and spiked with a volume of lab cultured FRNA, sufficient to achieve a concentration of 10^{7} - 10^{8} PFU.$(100\ mL)^{-1}$. Daily grab samples were taken from the feed tank (influent), activated sludge compartment (mixed liquor) and permeate. Densities of microorganisms were measured on all influent (C_{In}), mixed liquor (C_{ML}) and permeate (C_{Perm}) samples.

During trials one MBR was kept as a control, while three parallel MBRs were subjected to hazardous events. The hazardous events could be grouped into two categories according to the expected failure origin; severe feed water variation or process failure. Events originating from severe feed water variation included shock addition of chemicals (sodium chloride (NaCl), ammonia (NH_3) and 2, 4 di-nitro phenol (DNP)) and drastic chemical oxygen demand (High COD) loading. Process failure events included aeration loss (24 h), biomass washout, operation with defective membranes and fibre breakage. A summary of the event simulations and target real scenarios is included in Table 30.

Table 30- Summary of hazardous event simulations.

Feedwater Hazardous Events (Section 3.2)		
Event Name	Simulation description	Potential Real Scenario
NaCl	NaCl was added to activated sludge to achieve 20 $g.L^{-1}$	Seawater ingress
DNP	DNP was added to activated sludge to achieve 0.2 $g.L^{-1}$	Industrial waste discharge of an electron inhibiting compound.
NH_3	Ammonium carbonate was added to activated sludge to achieve 0.7 $g.L^{-1}$ as NH_3	Industrial waste discharge
High COD	1:1 mixture of glucose and glutamic acid was added to activated sludge to achieve 5 $g.L^{-1}$ as COD	Industrial waste discharge
Process Failure Hazardous Events (Section 3.3)		
Aeration failure	Aeration to the biomass and membrane compartment was switched off for 24 h.	Blower failure
Biomass washout	Direct filtration of sewage	Biomass washout due to wasting failure, severe rainfall or other loss of containment.
Operation with defective membranes	1 out of 4 mini-modules on the MBR rack was replaced with a mini-module with 5 – 50 µm defects.	Subtle, undetected abrasion of membrane fibres over time.
Fibre breakage	Up to 4 out of 120 fibres (c. 4%) were cut.	Severe integrity failure from a sharp foreign object

12.2.3 Calculation of Log Removal Values

Two different LRV were calculated, LRV_{MBR} (Equation 8) and LRV_{Bio} (Equation 9). LRV_{MBR} represented overall process removal by comparing influent and permeate microorganism densities. LRV_{Bio} compared influent and mixed liquor densities and was representative of removal due to the biopredation only.

$$LRV_{MBR} = \log_{10}\left|\frac{C_{In}}{C_{Perm}}\right| \quad \text{Equation 8}$$

$$LRV_{Bio} = \log_{10}\left|\frac{C_{In}}{C_{ML}}\right| \quad \text{Equation 9}$$

Where C_{In}, C_{ML} and C_{Perm} were the microorganism densities in the influent, mixed liquor and permeate respectively.

In order to assess the effect of a hazardous event on LRV, paired grab sample assays for the effected MBR were compared to a LRV distribution, constructed to represent removal during normal operation. C_{In}, C_{ML} and C_{Perm} densities were considered to represent normal operation for all days of control MBR operation, and for any operational days, during which MBRs were not subjected to hazardous events. Lognormal probability density functions (PDFs) were fit to cumulative microorganism densities, representing normal operation, with goodness of fit analysed by Root-Mean Squared Error. Monte Carlo simulation allowed the use of Equations 8 or 9, operating on microorganism PDFs, resulting in a PDF representing LRV_{MBR} and LRV_{Bio} for normal MBR operation. Monte Carlo simulation and PDF fitting was performed with @Risk software (Palisade Corporation, version 6.0) and Latin Hypercube sampling (using 10,000 iterations). Previous studies have used similar approaches in order to address limitations due to concentrations below permeate LOD and to account for performance variability (Olivieri *et al.* 1999, Khan *et al.* 2010, van den Akker *et al.* 2014). Lognormal distributions were previously shown as adequate for modelling parameters in treated and untreated wastewater (Oliveira *et al.* 2012). The 5th percentile and median of LRV PDFs represent conservative and optimistic performance respectively (Khan 2013). Accordingly, if a LRV was to drop below the 5th percentile after a hazardous event, then the impact on LRV was significantly different to normal operation.

12.2.4 Membrane Integrity Confirmation

Before and after trials membranes were subjected to pressure decay testing (PDT) in the laboratory. PDT was conducted with a starting pressure of 100 kPa in accordance with the method outlined in the US EPA membrane filtration guidance manual (USEPA 2005). Scanning electron microscopy (SEM) was enacted on samples of damaged and intact membrane fibres. Fibre samples were sputter coated with gold and imaged on a Hitachi S3400 SEM.

12.3 Results and Discussion

12.3.1 Benchmark LRV during normal operation

Samples representing normal operation were taken from the control MBR and for any operational periods before commencement of hazardous event simulations. LRV_{MBR} and LRV_{Bio} distributions were calculated for each indicator organism, corresponding to normal operation (Section 0). The number of samples taken across the trial period representative of normal operation is summarised in Table 31. For permeate samples, a ratio of assays above the LOD divided by the total number of assays was included. Influent and mixed liquor densities were above the LOD for all tested samples.

Table 31 - Number of samples combined from trials to create control set PDFs.

Organism	Number of Samples and Location		
	Influent	Mixed Liquor	Permeate Detects/Total
Clostridium perfringens	41	68	14/62
E. coli	34	73	26/71
Total Coliforms	36	73	41/63
FRNA Bacteriophage	17	40	29/31

A summary of the resulting LRV PDF parameters from this study compared to literature values is included as box plots in Figure 28. The 5th and 95th percentile were used for whiskers where more than 20 data points were cited, otherwise lowest and highest LRV was used.

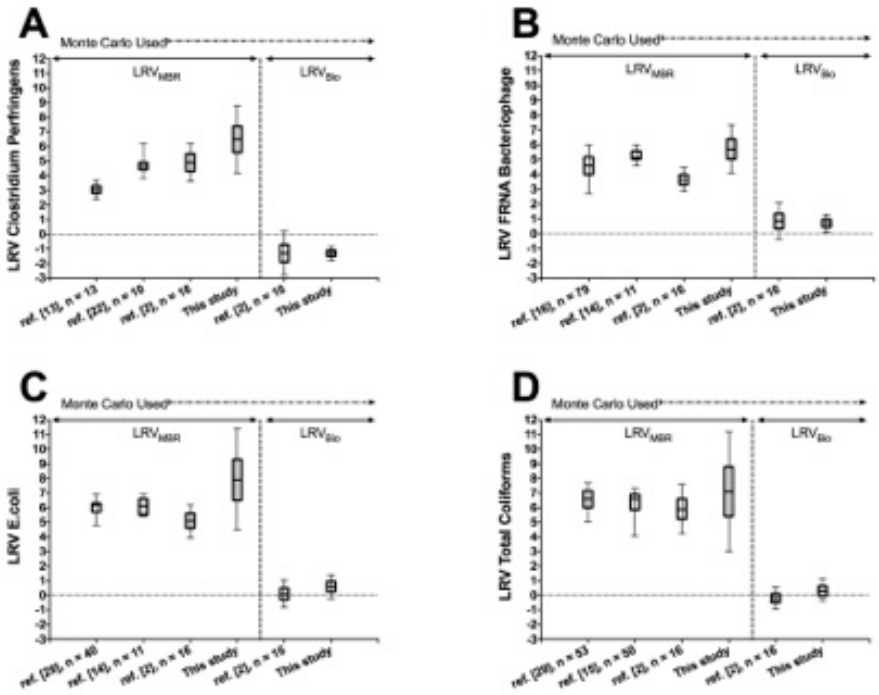

Figure 28 – Box plots of LRV_{MBR} and LRV_{Bio} from literature and this study for A) Clostridium perfringens, B) FRNA bacteriophage, C) E. coli and D) total coliforms. Monte Carlo simulation and PDF fitting was only used previously in ref (van den Akker et al. 2014). n is the number of LRV data points able to be extracted from referenced articles.

As with this study, PDF fitting of influent, mixed liquor and permeate densities was previously used to classify the performance of a full scale MBR (van den Akker *et al.* 2014). The box plots where PDF fitting and Monte Carlo simulation were used to obtain LRV are symmetrical (normally distributed), as expected when a log transform (Equations 8 or 9) is taken of a lognormal distribution. Skewness of LRV boxes in Figure 28 can be observed for all sets of data where previously reported LRVs were calculated with paired values. In particular, LRVs for EC, TC and FRNA from references (Severn 2003, Mosqueda-Jimenez *et al.* 2011, Francy *et al.* 2012, Hirani *et al.* 2012) were skewed to higher values, bounded by an upper limit, due to the influent concentration being removed to the detection limit a majority of the time. The use of PDF fitting and Monte Carlo simulation removed the skewness caused by method limitations, as it was possible to extrapolate to values below the permeate LOD. If the lognormal model is adequate to describe microorganism densities, as previously suggested (Olivieri *et al.* 1999, Khan *et al.* 2010, Oliveira *et al.* 2012, van den Akker *et al.* 2014), then a more realistic approximation of LRV was made possible with the approach used in this paper. The use of PDF fitting was particularly advantageous for quantifying LRV of larger microorganisms (CP and EC), a majority of permeate samples were below LOD (Table 31).

CP, EC and TC were previously investigated on pilot and full scale, exhibiting LRV_{MBR} of 3.0 – 6.2, 4.0 - 7.3 and 4.0 - > 7.7 respectively (Ottoson *et al.* 2006, Marti *et al.* 2011, Mosqueda-Jimenez *et al.* 2011, Francy *et al.* 2012, Hirani *et al.* 2012, van den Akker *et al.* 2014). LRV_{MBR} obtained in this study were in agreement with other studies around lower ranges (i.e. for this study 5th percentile LRV_{MBR} was CP: 4.1, EC: 4.5 and TC: 3.0), indicating that the 5th percentile is a robust measure of comparison. Median LRV_{MBR} in this study was higher by 0.5 to 2.8 log units for CP, EC and TC when compared to other studies. The higher median LRV_{MBR} was likely a combination of the new membranes utilised, operated at small scale, for short-term periods and integrity testing procedures ensuring better size exclusion than is possible for full scale plants. Although larger than the membrane pore size, TC were detected in the permeate for 41 of 63 samples and exhibited the highest treatment variability with a standard deviation of 2.5 log. TC have been reported to grow in permeate pipelines (DeCarolis *et al.* 2007). Additionally, TC concentration was the highest of all indicators in the influent, meaning initial TC contamination due to aerosols and growth in permeate pipeline was more likely than with other indicators. FRNA LRV_{MBR} for this study (4.1 – 7.3) corresponded more closely with the range reported in other studies (> 2.8 - > 6) (Severn 2003, Francy *et al.* 2012, van den Akker *et al.* 2014).

Median CP LRV_{Bio} (-1.3) in this work was in direct agreement with a previous study, although variability indicated by standard deviation, was lower (van den Akker *et al.* 2014). The negative LRV_{Bio} of CP indicated that this specific indicator accumulates within the bioreactor, under normal conditions. CPs ability to form spores and resist harsh environments may explain this accumulation (Venczel *et al.* 1997). However due to its large size, CP is easily rejected by the membrane. For EC and TC, accumulation within the bioreactor did not occur to the extent of CP, with a majority of LRV_{Bio} positive and a median removal of up to 0.6 log units within the biomass. FRNA LRV_{Bio} was always greater than zero, indicating appreciable removal of up to 1 log within the bioreactor. In this study activated sludge samples were analysed as a mixture without fractionation of solids and liquids; as a result, LRV_{Bio} is a measure representative of biological predation only. LRV_{MBR} is a combination of rejection of indicators, entrained in activated sludge flocs and supernatant, by the clean membrane and fouling layer. Figure 28 illustrates that LRV_{MBR} was 4 to 7 log units greater than LRV_{BIO} for all indicators, consistent with previous studies that measured the contribution of activation sludge (van den Akker *et al.* 2014, Chaudhry *et al.* 2015).

When assigning LRVs to unit operations in water recycling, a conservative and statistically valid approach is recommended by guidance documents; typically the 5th percentile LRV is used for this purpose (Khan 2013). The median is a resistant measure of the central tendency of a distribution, as it is less affected by extreme outliers when compared to the mean (Helsel *et al.* 2002). A system performing normally should exhibit removal performances centered around the median, but above the 5th percentile. In the following sections, a hazardous event was defined to have a significant effect in this study if LRV for an indicator reduced below the 5th percentile of the corresponding PDF.

12.3.2 Hazardous events originating from severe feed water variation

The impact of High COD, DNP, NH_3 and NaCl on LRV are presented in Figure 29. Sampling was conducted 15 minutes after shock loading on day 0. In Figure 29, LRV_{MBR} is shown with LRV_{Bio}, superimposed in order to highlight removal contribution due to biopredation compared to overall

removal by MBR. The difference between LRV_{MBR} and LRV_{Bio} is representative of removal across the membrane, including removal indicators entrained within activated sludge flocs.

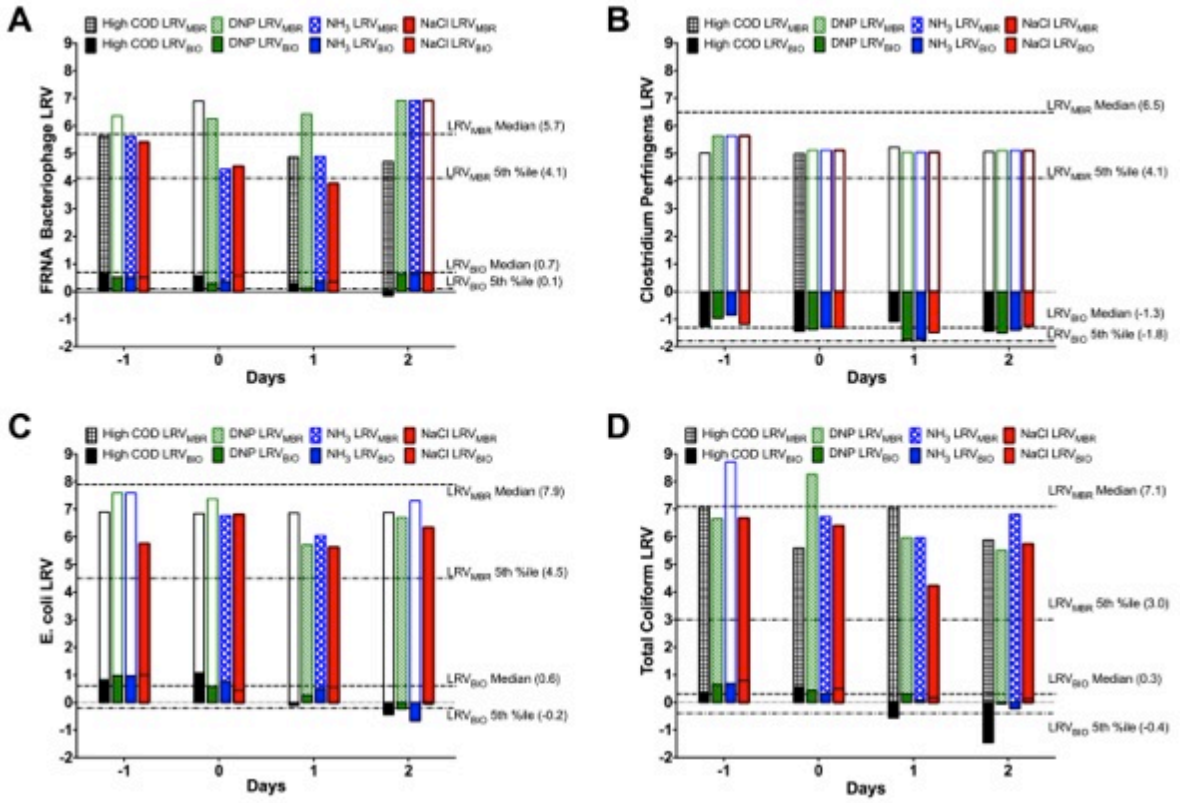

Figure 29 - Impact of feed water shocks enacted on day 0 to (A) FRNA, (B) CP, (C) EC and (D) TC LRV. LRV_{Bio} is shown superimposed on LRV_{MBR}. Interleaved bars from left to right represent high COD, DNP, NH_3 and NaCl shock loading for 1 day before and 2 days after shock loading. 5^{th} percentile and median control set values are shown for reference. Empty bars represent permeate assay below LOD.

12.3.2.1 High COD loading

For high COD loading a 1:1 ratio of glucose to glutamic acid was added in one load on day 0, in sufficient quantity to achieve a COD concentration of 5 $g.L^{-1}$ in the activated sludge. FRNA LRV_{Bio} was not immediately affected by addition of High COD. The first 1st day after shock loading, FRNA LRV_{Bio} reduced from 0.6 to 0.3, followed by further significant reduction to -0.2 by the 2^{nd} day. No recovery was evident within 3 days. Immediately following COD loading, FRNA LRV_{MBR} significantly increased to > 6.9 (i. e. no FRNA detected in the permeate). Due to the glutamic acid component of the COD loading, reactor pH reduced from the typical control range (6.8 ± 0.7) to 4.2 within 15 minutes. Given that the isoelectric point of FRNA bacteriophages is 3.9 it is likely that the drop in pH induced phage to phage and phage to sludge adsorption, increasing effective particle size, and as a result improved LRV_{MBR} (van Voorthuizen *et al.* 2001, Langlet *et al.* 2007, Langlet *et al.* 2008). FRNA LRV_{MBR} returned to normal within 1 day of high COD loading. Normalization of reactor pH indicated washout of a majority of glutamic acid within 1 HRT. The permeate DOC and COD concentrations (reported elsewhere (Trinh *et al.*)) had returned close to typical values of 5 - 10 $mg.L^{-1}$ within 3 HRT, indicating almost total washout of COD load within 3 days. Similar to FRNA, both EC and TC LRV_{Bio} displayed decreasing trends on the 1^{st} and 2^{nd} day following shock loading. EC LRV_{Bio} significantly decreased to -0.4 at 2 d while TC LRV_{Bio} reduced significantly to -0.6 and then -1.5 on days 1 and 2. EC LRV_{Bio} recovered by the 3^{rd} day to 0.6 and TC LRV_{Bio} showed signs of improvement, but was still below the 5^{th} percentile at -0.6. CP LRV_{Bio} was not significantly affected. COD loading under the conditions in this study was confirmed, as expected, to decreased removal capacity of the activated sludge. Significant fouling was triggered by the high COD shock, indicated by a TMP 2 to 3 times higher than the control MBR operating in parallel. Even with significant accumulation of indicator organisms in the activated sludge, COD loading did not significantly affect LRV_{MBR} for all indicators tested. The

increased fouling layer as a result of COD loading may have enhanced $LRV_{MBR,}$ reducing the effect of any loss of removal performance by the activated sludge.

12.3.2.2 DNP Loading

For DNP loading an equivalent mass of DNP was added on day 0 to achieve a concentration of 0.2 $g.L^{-1}$ in the activated sludge. Addition of DNP resulted in a bright yellow colour in the permeate, reducing in brightness over the three days. Permeate UV254nm absorbance measurements were typically 0.1 – 0.2 for control MBR permeate. Absorbance at UV254nm was 1.8, 2.0, 1.1 and 0.6 for permeate samples taken at 15 minutes, 1, 2 and 3 days after DNP shock loading respectively. Visual observation of colour and UV254nm absorbance suggests greater than 3 HRTs were required to washout DNP from the MBR. FRNA LRV_{Bio} reduced to the 5^{th} percentile of 0.1, 1 day after DNP shock loading. After 2 days, FRNA LRV_{Bio} recovered to the normal range at 0.6. Similar to FRNA, CP LRV_{Bio} reduced to the 5^{th} percentile of -1.8 after 1 day but showed recovery by the 2nd day. EC LRV_{Bio} displayed a decreasing trend following DNP addition, reducing to 0.3 and -0.2 over the 1^{st} and 2^{nd} day. No recovery of EC LRV_{Bio} was observed by the 3^{rd} day. TC LRV_{Bio} displayed the same decreasing trend as EC, but did not fall below the 5^{th} percentile LRV (-0.4) measured under normal conditions.

DNP loading did not significantly affect FRNA, EC, TC or CP LRV_{MBR}. Addition of DNP caused an increase in TMP of 200 to 300% relative to the control MBR, similar to high COD loading. Again, the fouling as a result of shock loading may have mitigated any loss of overall LRV due to a reduction in activated sludge performance.

12.3.2.3 Ammonia Loading

Ammonium carbonate was added as a single dose on day 0 in order to achieve an equivalent NH_3 concentration of 0.7 $g.L^{-1}$ in the activated sludge. FRNA LRV_{Bio} was not significantly affected, remaining above 0.1 for the trial duration. CP LRV_{Bio} did appear to decrease to the 5^{th} percentile 1 day after loading, but displayed recovery, returning close to the median at -1.4 by the 2^{nd} day. LRV_{Bio} for EC and TC displayed a declining trend, similar to DNP addition. EC LRV_{Bio} reduced significantly to -0.7 at 2 days after loading and did not recover by the 3^{rd} day. Although TC LRV_{Bio} reduced from 0.1 to -0.2, 1 and 2 days after NH_3 loading, removal did not fall below the 5^{th} percentile (-0.4). FRNA LRV_{MBR} reduced from 5.6 the day before NH_3 addition to 4.4 and 4.8 on days 0 and 1 respectively. FRNA LRV_{MBR} decrease was not significant, remaining above the 5^{th} percentile (4.1) for the trial duration. The increase to pH 8 due to addition of ammonia may have limited FRNA aggregation resulting in the minor LRV_{MBR} reduction (van Voorthuizen *et al.* 2001). LRV_{MBR} was not significantly affected for CP, EC and TC.

12.3.2.4 NaCl Loading

On day 0, 600 g of NaCl was added into the activated sludge in order to achieve a shock concentration of 20 $g.L^{-1}$. FRNA LRV_{MBR} was significantly reduced from 5.4, the day before NaCl addition, to 4.5 and 3.9 at 15 min and 1 d after shock. Addition of NaCl resulted in high levels of conductivity (i.e. 32 mS upon shockloading, measured in the permeate). In previous research increasing ionic strength, from 0 to 0.02 M as NaCl, was shown to enhance membrane rejection, in contrast to results from this study (van Voorthuizen *et al.* 2001). Other studies reported adsorption affinity of bacteria increased up to ionic strengths of 0.1M. At ionic strengths greater than 0.1 M, a reduction of adsorption affinity was noted relating to the molar concentration of cations (Stevik *et al.* 2004). By addition of 20 $g.L^{-1}$of NaCl, ionic strengths for shock trials increased equivalent to 0.3 M. Increase of ionic strength to such high levels may have reduced virus adsorption to sludge, resulting in transfer across the membrane as a result of decreased effective particle size. FRNA LRV_{MBR} recovered 2 days after shock loading, corresponding to return of permeate conductivity to typical levels of 1 – 3 mS and passage of 2 HRTs. As with DNP and COD shock-loading, additional fouling was triggered by addition of NaCl. High reactor TMPs, 2 to 5 times the parallel control MBR on days after the shock, may also have assisted recovery of LRV_{MBR}. LRV_{Bio} and LRV_{MBR} were not significantly affected by NaCl shock loading for CP, EC and TC.

12.3.3 Hazardous events originating from process failures

The impact of operation with defective membranes, aeration loss (24 h) and direct sewage filtration on LRV are presented in Figure 30. Operation of MBRs with defective membranes and direct sewage filtration was started on day 0, sampling was conducted 15 minutes after start up. For aeration loss,

blowers were was switched off 15 minutes before sampling on day 0 and recommenced 15 minutes before sampling on day 1.

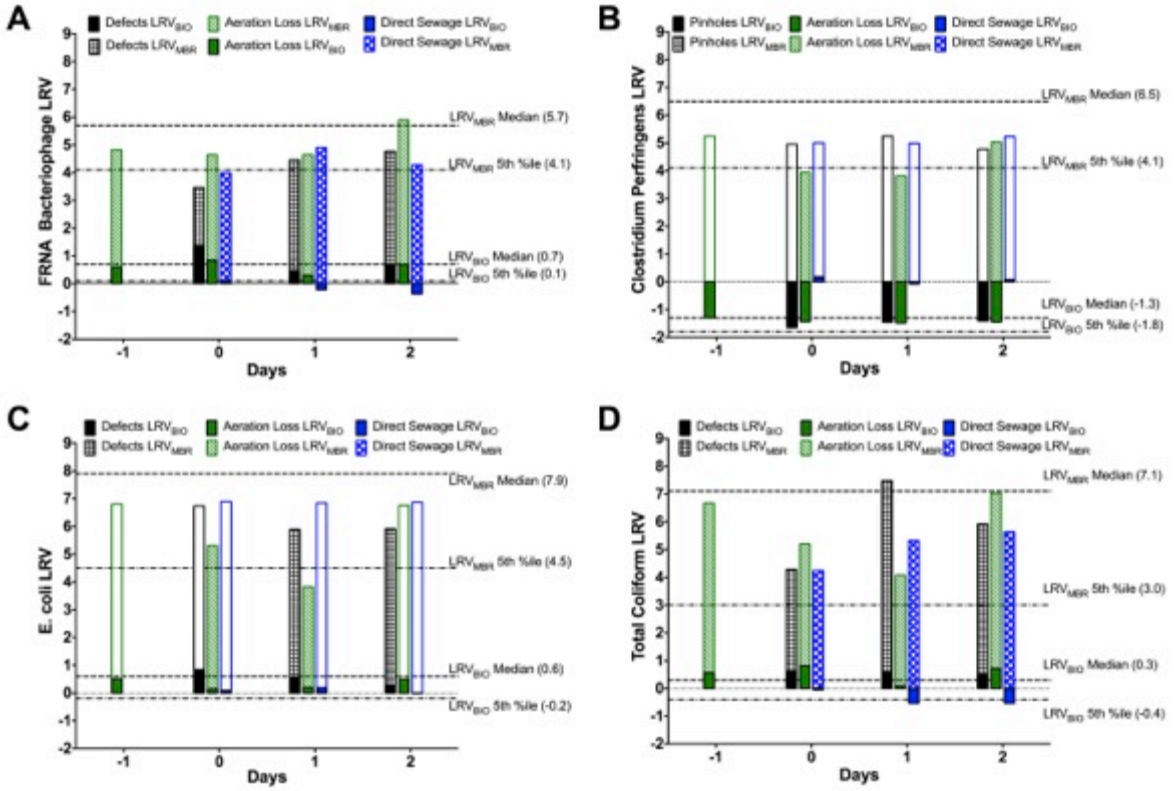

Figure 30 - Impact of defective membranes, aeration loss and direct sewage filtration to (A) FRNA, (B) CP, (C) EC and (D) TC LRV. LRV_{Bio} is shown superimposed on LRV_{MBR}. Interleaved bars from left to right represent defective membranes, aeration loss and direct sewage filtration. Hazardous events were started on day 0. 5th percentile and median control set values are shown for reference. Empty bars represent permeate assay below LOD.

12.3.3.1 Defective Membranes

For the trial with defective membranes, one out of four intact membrane modules was replaced with an equivalent, created with industrially damaged fibres. The defective fibres were taken from a membrane autopsy conducted after the module had been in service for 1 year, and been damaged by the presence of sharp foreign objects. SEM was used to identify defects in the damaged fibres, ranging from 5 – 50 µm in diameter (Figure 31). From SEM observation, a majority of defects appeared to be smaller, in the order of 5 µm. Depending on calculation assumptions, a PDT at 100 kPa should have sufficient resolution to detect defects with diameter greater than 0.4 – 3.0 µm (USEPA 2005). Typical pressure decay rate (PDR) for intact membrane modules was 0.5 kPa.min^{-1} and modules were not used in trials if the PDR was > 1.0 kPa.min^{-1}. PDT on the damaged module alone and combined in a rack with 3 intact modules yielded PDRs of 20.0 and 16.5 kPa.min^{-1} respectively. PDT confirmed that numerous defects had penetrated the membrane skin. The PDR of the defective membrane indicated a high level of damage and significantly increased the PDR of the combined membrane rack, when tested with 3 intact modules.

As expected, LRV_{Bio} for all indicators was unaffected by filtration with defective membranes, centered about the median of normal operation LRV PDFs. FRNA LRV_{MBR} was 3.5, significantly below the 5th percentile (4.1) upon startup. The 1st and 2nd day after start up, FRNA LRV_{MBR} continued to increase to 4.5 to 4.8, recovering above the 5th percentile in less than 1 day. LRV_{MBR} for EC, TC and CP was not significantly affected when compared to LRV PDFs of normal operation. PDT was effective at identification of membrane integrity damage. However, the skin defects appeared to be easily shielded by activated sludge flocs, resulting in stabilisation of FRNA LRV_{MBR} within 24 h. Shielding of defects appeared more rapid and effective at protection against transfer of larger indicators EC, TC and CP, with no significant change in LRV upon startup.

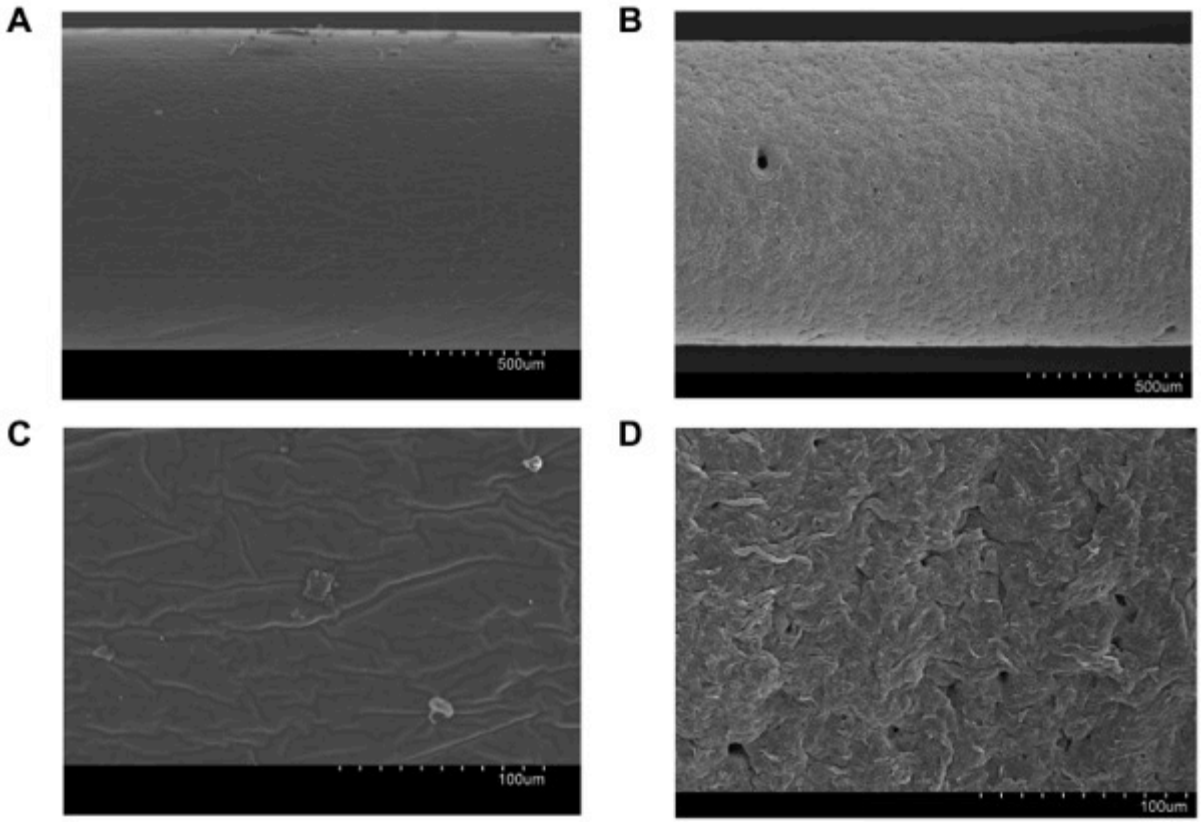

Figure 31 - SEM comparison of intact (A & C) and damaged membrane fibres (B & D). Magnification increases from top to bottom.

12.3.3.2 Aeration Loss

For normal operation, the activated sludge was aerated at 15 min on/off cycles. Continuous aeration of the membrane compartment was performed with a second blower to reduce fouling. In order to simulate complete blower failure, air supply for the membrane compartment and activated sludge was isolated from the reactor for 24 hours, starting on day 0. FRNA, EC and TC LRV_{Bio} decreased to 0.3, 0.1 and 0.1 respectively after 24 hours without aeration. 1 day after return of aeration, FRNA, EC and TC LRV_{Bio} recovered to normal levels. LRV_{Bio} for all indicators did not reduce significantly, remaining above the 5th percentile control value for the duration of the trial. FRNA LRV_{MBR} was not affected by aeration loss. Both EC and TC LRV_{MBR} declined falling to 5.3 and 5.2 respectively within 15 min of aeration being switched off. After 24 hours without aeration EC LRV_{MBR} was significantly reduced to 3.8, below the 5th percentile (4.5). TC LRV_{MBR} also reduced after 24 hours without aeration to 4.0. Given the substantial variability in TC removal (i.e. 5th percentile 3.0), a significant change could not be concluded. CP LRV_{MBR} was immediately and significantly reduced to 3.8 for the 24 h that aeration was off. Within 24 h after aeration was returned CP, TC and EC LRV_{MBR} returned to normal.

The loss of containment of CP, EC and TC was unexpected, especially when no significant change occurred to FRNA LRV_{MBR}. Additionally, CP was not detected in the permeate of the defective membrane trials. The installed membranes for aeration loss passed integrity testing with, a PDT of 0.5 kPa.min^{-1}, at a test pressure of 100 kPa. The test pressure required to detect a defect of 1 µm is approximately 50 - 300 kPa, depending on more or less conservative factors are used in the calculation (USEPA 2005). Hence there is a chance defects large enough for EC, TC and CP to pass through may have remained undetected by PDT. The day after the aeration was switched off, settling of the solids was observed in the membrane compartment as the only agitation in that section of the MBR was due to continuous aeration. The mixed liquor remained suspended due to a stirrer in the activated sludge compartment. CP densities are higher in the bioreactor compared to the influent (i.e. Median LRV_{BIO} = -1.3). Higher densities of pathogens against the membrane increase the likelihood for transmission. The possibility of undetectable membrane defects, combined with the reduced membrane shielding as a result of biomass settling, may have resulted in the overall reduction of CP,

EC and TC LRV_{MBR}. FRNA LRV_{MBR} may not have been similarly affected as previous studies have shown a majority of virus particles associate with activated sludge flocs (Xagoraraki *et al.* 2014). As a result, the unshielded membrane would have been exposed to lower densities of FRNA in the supernatant, due to a majority of FRNA remaining attached to settled activated sludge. The hypothesis regarding settling was further supported by the quick recovery of EC, TC and CP LRV_{MBR} (< 24h) following restart of aeration and consequent re-suspension of activated sludge. Membrane permeability for aeration loss was high and identical to the control, falling from 5.9 to 3.1 $L.m^{-2}.h^{-1}.kPa^{-1}$ for the 6 day trial and indicated a low fouling level. As a result, the breakthrough during aeration loss is expected to be worse than for a membrane, operated for longer term, with a higher level of irreversible fouling.

12.3.3.3 Biomass Washout

Biomass washout was conservatively simulated by filtration of sewage, without the presence of activated sludge. FRNA LRV_{Bio} significantly declined following startup from 0.1 to -0.2. No recovery was observed within 3 days after start up, with FRNA LRV_{Bio} remaining below -0.2, showing signs of accumulation in the bioreactor, without the presence of activated sludge. EC LRV_{Bio} was low (0.1 ± 0.1) but not below the 5th percentile (-0.2). TC LRV_{Bio} significantly declined from 0 to -0.5 for the 1st and 2nd day after the trial start. By the 3rd day, TC LRV_{Bio} had recovered to the median of the normal operation PDF (0.3). CP LRV_{Bio} was substantially improved compared to the median (-1.3), centered about 0, as insufficient volume was processed during the trial for CP to accumulate to normal densities typical in activated sludge. FRNA LRV_{MBR} was 4.0, below the 5th percentile (4.1) on the 1st day. FRNA LRV_{MBR} improved, to above the 5th percentile at 4.9, 4.2 and 4.8, over the 1st, 2nd and 3rd days respectively. CP, TC and EC LRV_{MBR} were not significantly affected. Direct filtration of sewage resulted in fouling rates 40 times greater than the typical control range (Trinh *et al.*). The stabilisation of FRNA LRV_{MBR}, even after significant accumulation within the bioreactor, was likely due to development of a tight fouling layer aiding rejection.

12.3.3.4 Fibre Breakage

Using a scalpel, fibres were cut as close as possible to the suction end of a single mini-module. Fibre breakage trials were conducted over 4 d. On day 1 the MBR was operated normally. On the 2nd day, 3 out of 120 fibres in the reactor were cut. Results from day 2 appeared to show no significant change in LRV. In order to investigate the lack of breakthrough, sampling was conducted at 0 and 5 minutes after a backflush (5 min, at – 30 $L.m^{-2}.h^{-1}$) and after cutting a 4th fibre on the 3rd day. On day 4 samples were taken normally, 24 hours after a total of 4 out of 120 fibres were cut. LRV_{MBR} during fibre breakage experiments is shown in Figure 32. Total MBR permeate limited sample volume in 1 minute to 25 mL. This necessitated use of smaller analysis volumes for 0 and 5 minute samples, resulting in LOD of 100 $CFU.(100\ mL)^{-1}$, 100 $PFU.\ (100\ mL)^{-1}$ and 2000 $CFU.(100\ mL)^{-1}$ for CP, FRNA and EC and TC respectively. Normal LOD of 10 PFU/100 mL for FRNA and 1 CFU/100 mL for CP, EC and TC applied for all other samples.

15 min after cutting 3% of fibres, LRVMBR for all indicators was not significantly affected. 1 day later, after observing negligible change in LRVMBR, the MBR was sampled at 0 and 5 minutes after backflush. Following backflush, LRVMBR for CP, TC and FRNA dropped significantly to 1.2, 2.5 and 2.7 respectively. 5 min later LRVMBR for CP, TC and FRNA had recovered to > 3.2, > 4.7 and 5.9. Permeate turbidity was recorded as 24 NTU 0 min after backflush, recovering to 0.07 NTU at 5 min. Given the quick recovery of turbidity a 4th fibre was cut, increasing the overall damage rate to approximately 4%. Immediately after cutting a 4th fibre CP, TC and FRNA LRVMBR significantly reduced to 1.5, 3.0 and 3.1 respectively. After 5 min, CP, TC and FRNA LRVMBR had increased to > 3.2, > 4.7 and 5.6. Change in turbidity corresponded with microorganism breakthrough after the 4th cut fibre, increasing to 12 NTU immediately after cutting and recovering to 0.05 NTU at 5 min. EC LRVMBR was > 3.6, not detected above the smaller sample LOD of 100 CFU.(100 mL)-1 in the permeate, at 0 or 5 min after backflushing and cutting a 4th fibre. 15 min after the 4th cut fibre, CP, EC, TC and FRNA LRVMBR had recovered above the 5th percentile LRVMBR at > 5.2, 6.6, 6.5 and 5.8 respectively. Samples taken on day 4, 24 hours after a total damage rate of 4% fibre breakage, indicated that LRVMBR remained stable, with all indicators above the 5th percentile. Examination of the cut module post experiment revealed that suspended solids plugs, approximately 10 mm in length had formed within the hollow fibre lumen. The suspended solids plugs were displaced from the lumen by application of 30 kPa air pressure.

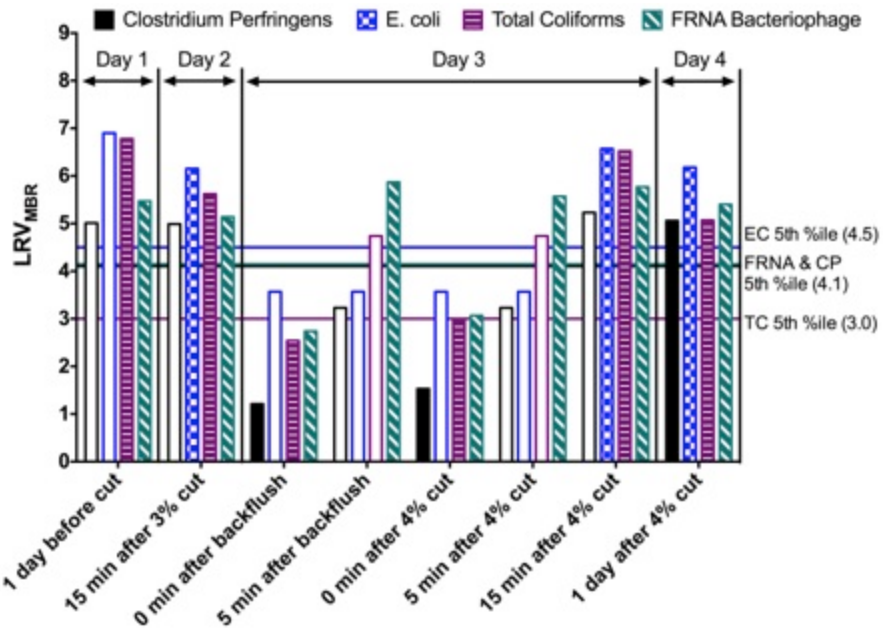

Figure 32 – From left to right CP, EC, TC and FRNA LRV_{MBR} for fibre breakage trials. Open bars denote permeate densities below limit of detection.

As expected, fibre breakage quickly caused a significant reduction in LRV_{MBR}. However, under the conditions of this study, suspended solids plugging of the hollow fibre lumen occurred rapidly, with LRV effectively restored within 5 to 15 minutes. The quick formation of plugs explained why no LRV change was discernable when sampling 15 min after cutting 3% of the fibres on day 2. The plugs were reversible, displaced when compressed air was applied at 30 kPa or backflushed at 30 $L.m^{-2}.h^{-1}$ for 5 minutes.

12.4 Conclusions

Under normal operational conditions in this study, LRV_{Bio} was 4 to 7 log lower than LRV_{MBR}, indicating the majority of removal occurs across the membrane. In general, events simulating severe feed water variation significantly reduced LRV_{Bio}. However, impact on overall removal (LRV_{MBR}) was not significant. NaCl shock was an exception, significantly reducing virus retention, indicated by FRNA bacteriophages.

The membrane and activated sludge behave synergistically in an MBR. Fouling as a result of activated sludge stress may have mitigated breakthrough across the membrane during feed water variation events. Settling of sludge, as a result of aeration loss, appeared to allow significant passage of suspended microorganisms through exposed membrane area. Small but numerous defects in the membrane skin could be shielded by activated sludge, resulting in enhanced removal. As expected, fibre breakages significantly reduced LRV, but under the conditions tested, could rapidly plug with sludge resulting in recovery to normal removal conditions. Combinations of hazardous events (e.g. integrity failure + severe feed water variation reducing biological performance) may result in catastrophic system failure. Otherwise, MBR performance was resilient to events tested in this study.

This paper quantified the consequences of hazardous events. To that end, more informed and efficient risk management of MBR can be achieved through prioritisation of control strategies, with respect to hazardous event consequence. According to the results of this work, monitoring of membrane integrity will assure a majority of log removal capability ($LRV_{MBR} > LRV_{BIO}$). Monitoring source water quality to ensure pH and conductivity remain within a specified envelope is secondary but could ensure more stable entrainment of viruses. Likelihood of occurence, in addition to consequence, are both required in order to assess the risk of a hazardous event. Scoping other possible hazardous events to MBR and also quantification of the likelihood of failures would further complement this work.

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Appendix 1: Overview matrix for Hazardous Events to MBR LRV

Event	Description	Parameters Monitored[i]	Change to LRV[ii, iii, iv]	Time to Recovery[v]	Comments
1. High COD Loading[vi]	Equivalent concentration of 5 g/L COD (1:1 Glucose: Glutamic Acid Solution) added to bioreactor	Typical	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = N/A EC: LRV_{MBR} = N/A EC: LRV_{BIO} = -0.5, within 48 hr. TC: LRV_{MBR} = N/A TC: LRV_{BIO} = -1.5, within 24 hr. FRNA: LRV_{MBR} = initial improvement (< 24hr) due to pH drop, then N/A FRNA: LRV_{BIO} = -0.2, within 24 hr.	EC: LRV_{BIO}, 72 hr TC: LRV_{BIO}, > 72 hr FRNA: LRV_{BIO} > 72 hr	CST, DOC removal and COD removal recovered 2 – 3 days after shock loading. Fouling rate (TMP/time) was initially increased following shock loading. Although accumulation of EC, TC and FRNA was observed within the reactor, the net effect on LRV_{MBR} was negligible. The decrease in sludge filterability and resultant fouling was thought to preserve overall LRV.
2. DNP shock load[vi]	Equivalent concentration of 200 mg/L 2,4 –dinitrophenol was added to bioreactor	Typical	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = reduced to 5th %ile of -1.8 after 24 hr EC: LRV_{MBR} = N/A EC: LRV_{BIO} = reduced to -0.4 within 72 hr TC: LRV_{MBR} = N/A TC: LRV_{BIO} = reduced to -0.4 within 72 hr FRNA: LRV_{MBR} = N/A FRNA: LRV_{BIO} = reduced to 5th %ile of 0.1 after 24 hr	CP: LRV_{BIO}, 48 hr EC: LRV_{BIO}, > 72 hr TC: LRV_{BIO}, > 72 hr FRNA: LRV_{BIO}, 48 hr	COD and DOC removal were reduced for > 48hr. Overall LRV were not effected. LRV_{BIO} did not drop substantially below 5th %ile.
3. Ammonia[vi]	Equivalent concentration of 700 mg/L NH_3 was added to bioreactor	Typical	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = reduced to 5th %ile of -1.8 after 24 hr EC: LRV_{MBR} = N/A EC: LRV_{BIO} = reduced to -0.8 in 72 hr TC: LRV_{MBR} = N/A TC: LRV_{BIO} = reduced to -0.6 in 72 hr FRNA: LRV_{MBR} = N/A FRNA: LRV_{BIO} = N/A	CP: LRV_{BIO}, 48 hr EC: LRV_{BIO}, > 72 hr TC: LRV_{BIO}, > 72 hr	CST and fouling rate significantly increased recovery > 48 hr. Fouling increase halted by relaxation 24 hr after shock. COD and DOC removal decreased at 24 hr but recovered within 48 hr. No significant effect to overall LRV

Event	Description	Parameters Monitored	Change to LRV	Time to Recovery	Comments
4. NaCl[vi]	Equivalent concentration of 20 g/L NaCl was added to the bioreactor	Typical + Permeate conductivity	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = N/A EC: LRV_{MBR} = N/A EC: LRV_{BIO} = reduced to -0.6 in 72 hr TC: LRV_{MBR} = N/A TC: LRV_{BIO} = reduced to -0.7 in 72 hr FRNA: LRV_{MBR} = reduced to 3.9 in 24 hr FRNA: LRV_{BIO} = N/A	EC: LRV_{BIO}, > 72 hr TC: LRV_{BIO}, > 72 hr FRNA: LRV_{MBR}, 48 hr	DOC removal decreased, recovering within 48 hr. COD removal decreased immediately, recovering in 24 hr. FRNA overall LRV decreased significantly. Permeate conductivity normalized within 48 hr, corresponding to recovery of FRNA: LRV_{MBR} suggesting an relationship between salt concentration on FRNA rejection.
5. Fibre Breakage[vi]	3% of fibres cut on day 0, On day 1, backflushed, then total 4% of fibres cut.	Typical + Turbidity + Pressure Decay Testing	CP: LRV_{MBR} = reduced to 1.2 immediately after backflush CP: LRV_{BIO} = N/A EC: LRV_{MBR} = reduced to > 3.5 immediately after backflush (LOD higher due to smaller sample volume available. EC: LRV_{BIO} = N/A TC: LRV_{MBR} = reduced to 2.5 immediately after backflush TC: LRV_{BIO} = N/A FRNA: LRV_{MBR} = reduced to 2.7 immediately after backflush FRNA: LRV_{BIO} = N/A	CP: LRV_{MBR}, 15 min EC: LRV_{MBR}, 15 min TC: LRV_{MBR}, 15 min FRNA: LRV_{MBR}, 15 min	No significant decrease in LRV_{MBR} was observed 15 minutes after cutting 3% of fibres. The following day a significant reduction in LRV_{MBR} was seen for samples taken immediately after backwash and cutting a 4th fibre. Turbidity and LRV_{MBR} recovery appeared to correlate. Fibre internal diameter plugging was confirmed by post trial module examination. Fibre plugging was partial 5 min after cutting and complete 15 min after cutting. Pressure decay testing confirmed substantial module damage, as it removed the fibre plugs.
6. Defective Fibres[vi]	1 of 4 modules in the rack was replaced with a minimodule created from membrane fibres featuring 5 – 50 µm defects.	Pressure Decay Testing + Typical	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = N/A EC: LRV_{MBR} = N/A EC: LRV_{BIO} = N/A TC: LRV_{MBR} = N/A TC: LRV_{BIO} = N/A FRNA: LRV_{MBR} = 3.5 upon start up FRNA: LRV_{BIO} = N/A	FRNA: LRV_{MBR}, 24 hr	Pressure decay testing confirmed substantial damage, with the PDT of the single compromised module contributing significantly to the PDT of the combined rack. FRNA overall LRV was the only indicator significantly effected. Within 24 hr it appeared that the membrane defects were shielded as FRNA LRV returned to above the 5th %ile. FRNA LRV continued to increase for 72 hr following startup.

Event	Description	Parameters Monitored	Change to LRV	Time to Recovery	Comments
7. Biomass washout[vi]	MBR was filled with sewage. Direct filtration of sewage was carried out.	Typical	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = improved, no accumulation LRV centred around 0. EC: LRV_{MBR} = N/A EC: LRV_{BIO} = N/A TC: LRV_{MBR} = N/A TC: LRV_{BIO} = -0.5 at 24 hr FRNA: LRV_{MBR} = 4.0 upon start up FRNA: LRV_{BIO} = -0.3	TC LRV_{BIO}, 72 hr FRNA: LRV_{MBR}, 24 hr FRNA: LRV_{BIO}, > 72 hr	TC and FRNA LRV_{BIO}, showed initial accumulation following start up. Biomass contribution to degradation of FRNA did not recover during the trial. TC recovered within 72 hr. COD and DOC removal did not recover to the control mean within 48 hr, indicating a negligible biological activity. Fouling rate was significantly increased relative to the control. FRNA:LRV_{MBR} recovered quickly due to fouling.
8. Aeration loss[vi]	Aeration to activated sludge and membrane compartment were switched off for 24 hr	Typical	CP: LRV_{MBR} = reduced to 3.8 after 24 hr CP: LRV_{BIO} = N/A EC: LRV_{MBR} = reduced to 3.8 after 24 hr EC: LRV_{BIO} = N/A TC: LRV_{MBR} = N/A TC: LRV_{BIO} = N/A FRNA: LRV_{MBR} = N/A FRNA: LRV_{BIO} = N/A	CP: LRV_{MBR}, 24 hr EC: LRV_{MBR}, 24 hr	Reductions in LRV_{MBR} were observed for all indicators. Loss of aeration resulted in settling of the activated sludge in the membrane compartment. It is thought that this settling left micro defects unshielded resulting in passage of indicators. 24 hr after return of aeration, and re-suspension of sludge rejection had improved for all indicators.
9. 4 day shutdown and Biomass washout (full scale)[vii]	Diffuser maintenance at a full-scale plant necessitated drain down of the aerobic compartment. Maintenance lasted 4 days. As a result 50% of biomass was lost on restart and replaced with wastewater.	DOC Turbidity TMP Flux MLSS/VSS CST EC TC CP FRNA Somatic Coliphage (SC)	CP: LRV_{MBR} = N/A CP: LRV_{BIO} = showed improvement > 95th %ile, returned to normal in 2 weeks EC: LRV_{MBR} = reduced to 4.9 EC: LRV_{BIO} = reduced to 0.1 TC: LRV_{MBR} = reduced to 5.6 TC: LRV_{BIO} = N/A FRNA: LRV_{MBR} = N/A FRNA: LRV_{BIO} = N/A SC: LRV_{MBR} = reduced to 2.4 SC: LRV_{BIO} = N/A	EC: LRV_{MBR}, > 5 d EC: LRV_{BIO}, > 5 d TC: LRV_{MBR}, 5 d SC: LRV_{MBR}, > 5 d	Biomass washout would appear to be a significant hazardous event, taking longer than 5 days to recover to 5th percentile LRVs for Bacteria (EC and TC) and Virus indicators (SC). *Clostridium perfringens*, removed primarily by size exclusion, displayed no sensitivity to biomass washout. CP LRV_{BIO} appeared improved due to washout of accumulated CP. DOC removal, CST and MLSS were significantly affected requiring longer than 5 d to return to normal levels.

Event	Description	Parameters Monitored	Change to LRV	Time to Recovery	Comments
10. Weekly chemically enhanced backwash (CEB)[v ii]	Samples were taken before and after weekly maintenance cleaning with NaOCl in order to assess the effect on LRV.	Total Cl_2 Turbidity EC TC CP FRNA SC	CP: LRV_{MBR} = N/A EC: LRV_{MBR} = N/A TC: LRV_{MBR} = slight improvement FRNA: LRV_{MBR} = N/A SC: LRV_{MBR} = N/A	Normalization within 2 hr	Up to 35 mg/L of total Cl_2 remained after CEB, requiring 20 min to flush out. This disinfectant dosage was thought to protect against any possible breakthrough during this period. Turbidity required 30min – 1 hr to return to less than 0.2 NTU. At 2 hr, no significant effect was seen to LRV.
10. Yearly clean in place (CIP)[v ii]	Samples were taken for 6 days after a 24 hr CIP with NaOCl	Total Cl_2 Turbidity TC EC CP	CP: LRV_{MBR} = N/A EC: LRV_{MBR} = reduced to 5.2 TC: LRV_{MBR} = reduced to 4.9	EC: LRV_{MBR}, 5 d TC: LRV_{MBR}, 5 d	Virus methods were not established at the time of the event. Bacteria LRV required 5 d to return above the 5th %ile LRV.Turbidity required 4 hr to stabilize to the typical value of 0.1 NTU

[i] TMP = Trans membrane pressure, COD = chemical oxygen demand, DOC = dissolved organic carbon, CST = capillary suction time of activated sludge, MLSS/VSS = mixed liquor suspended solids/volatile suspended solids, FRNA = FRNA bacteriophage, EC = *E. coli*, TC = total coliforms and CP = *Clostridium perfringens* were monitored for all trials. Any additional parameters are reported.

[ii] Change to LRV is considered N/A if drop in LRV did not reduce below 5th percentile from control set (SP1 Milestone 3 – Section 3.2.2)

[iii] $LRV_{MBR} = Log_{10}(C_{influent}/C_{permeate})$, representing overall process removal

[iv] $LRV_{MBR} = Log_{10}(C_{influent}/C_{mixed\ liquor})$, representing removal due only to the activated sludge

[v] Time until LRV returns above 5th %ile LRV

[vi] Tested on 30L MBR pilots, batch fed daily with screened and settled sewage from a primary WWTP (SP1 Milestone 3 – Section 2.2.2)

[vii] Tested on 100 kL/day full scale MBR (SP1 Milestone 3 – Section 4)

Appendix A:

Membrane Bioreactor Validation Protocol

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Abbreviations

BOD:	Biochemical oxygen demand
CCL:	Critical control limit
CEB:	Chemically enhanced backwash
CIP:	Clean in place
C_{In} :	The density of a microorganism in the influent
COD:	Chemical oxygen demand
CP:	*Clostridium perfringens*
C_{Perm}:	The density of a microorganism in the permeate
CST:	Capillary suction time
DIT:	Direct integrity testing
DO:	Dissolved oxygen
DOC:	Dissolved organic carbon
EC:	*Escherichia coli*
FRNA:	FRNA bacteriophages
F/M ratio:	Food to microorganism ratio
HRT:	Hydraulic retention time
IEP:	Iso-electric point
LOD:	Limit of detection
LRC:	log removal credit
LRV:	Log removal value
LRV_{CCL}:	The log removal value at a particular critical control limit
$LRV_{C\text{-}test}$:	The log removal value determined from challenge testing
MBR:	Membrane bioreactor
MF:	Microfiltration
MFGM:	Membrane Filtration Guidance Manual
MLSS:	Mixed liquor suspended solids
MLVSS:	Mixed liquor volatile suspended solids
NaOCl:	Sodium hypochlorite
PDT:	Pressure decay test
QA/QC:	Quality assurance/quality control
SC:	Somatic coliphages
SRT:	Solids retention time
TMP:	Trans-membrane pressure
UF:	Ultrafiltration
USEPA :	United States Environmental Protection Agency
VDoH:	Victorian Department of Health

1. Background

Membrane bioreactors (MBRs) are a suitable technology for nutrient removal, in place of activated sludge, where plant footprint is constrained. Due to incorporation of a membrane in place of a clarifier, MBRs also achieve moderate to high disinfection of pathogens consistently.

Micro (MF) and ultrafiltration (UF) membrane systems are often validated through use of direct integrity testing (DIT), e.g. pressure decay testing. Although MBRs utilise MF or UF membranes, most MBR systems are not equipped for DIT. In addition, the comparatively harsh conditions as opposed to direct filtration mean there is potentially more difficulty passing DIT in MBR. If DIT is proposed, then the MBR could be validated as a MF or UF system, ignoring any contribution of the activated sludge, in accordance with the USEPA membrane filtration guidance manual (USEPA 2005). This document details the approach for validation of an MBR without the use of DIT.

This document has been prepared to provide guidance for validation of MBRs for pathogen reduction in water reuse schemes. This document used Chapter 7 of the Victorian Department of Health's Guidelines for validating treatment processes for pathogen reduction (VDoH 2013), as a basis. Uncertainties listed in VDoH 2013 were addressed via experimental research activities and/or literature review. In addition, several previous MBR validation reports and recycled water quality management plans were critically assessed, in order to establish current practice.

This document sequentially covers the following elements:

1. Identification of the mechanisms of pathogen removal by MBRs.
2. Identification of target pathogens, and appropriate surrogates, that are the subject of the validation study.
3. Identification of the influencing factors that affect the efficacy of MBRs to reduce target pathogens.
4. Identification of operational monitoring parameters that can be measured continually and that will relate with the reduction of the target pathogen.
5. Description of a validation methodology to demonstrate the capability of MBRs.
6. Description of a method to collect and analyse data to formulate evidence based conclusions.
7. Description of a method to determine critical limits as well as an operational monitoring and control strategy
8. Description of a method to determine the log removal value (LRV) for each pathogen group in MBRs performing within defined critical limits
9. Guidance on triggers for re-validation or additional verification testing.

This document is a working draft. Further work and supporting material are under construction to ensure recommendations are consistent with current knowledge and best practice. A companion document with more detailed justification in appendices is cross-referenced through this draft guideline.

2. Identification of Pathogen Removal Mechanisms

The primary mechanisms for pathogen removal in MBR are size exclusion, entrainment within activated sludge flocs or membrane fouling layer, and biological predation. Previous studies have indicated the importance of the fouling layer and entrainment in sludge flocs in aiding removal of viruses (0.01 – 0.1 μm), that are smaller than the typical membrane pore size (0.04 – 0.4 μm) in MBRs (Hai *et al.* 2014). Protozoa and bacteria, typically larger than the membrane pores, are naturally removed to a larger extent by size exclusion, regardless of level of fouling. The relative contribution of each mechanism to

LRV is known to be pathogen or indicator specific. Pathogen removal mechanisms were reviewed and reported in Appendix A.

3. Identification of Target Pathogens

Table 1 shows target reference pathogens and relevant process indicator microorganisms chosen for MBRs. Reference pathogens were adapted from recently released NSW Office of Water, water recycling guidelines (NSW_OFW 2015) and are consistent with the Australian Guidelines for Water Recycling (NRMMC/EPHC 2006). The exception is that the reference virus is now updated to an amalgam of rotavirus and adenovirus.

Process indicators were selected based on current practice from previous validation reports, critical assessment of literature and consideration of the current Victorian Validation Guidelines (VDoH 2013). Direct measurement of pathogenic species in wastewater is often not feasible due to low and highly variable concentrations and complex analysis procedures (Antony *et al.* 2011). As a result, process indicator organisms are often chosen as surrogates for pathogens. A suitable indicator organism should be chosen such that it displays correlated or more conservative removal than the target pathogen (VDoH 2013). More background on the justification of these recommendations is available in the following sections.

Table 1 - Target Reference Pathogens and Process Indicators for MBRs

Hazard	**Reference Pathogen**	**Process Indicators**
Protozoa	*Cryptosporidium parvum*	*Clostridium perfringens*
Bacteria	*Campylobacter*	*Escherichia coli*
Virues	Amalgam of rotavirus and adenovirus	Somatic coliphages and FRNA bacteriophages

3.1. Viruses

FRNA bacteriophages (FRNA) have been investigated in several previous studies of log removal in MBR (Severn 2003, Ottoson *et al.* 2006, Hirani *et al.* 2010,

Pettigrew *et al.* 2010, Francy *et al.* 2012, Hirani *et al.* 2012, van den Akker *et al.* 2014). FRNA was selected as an indicator of virus removal performance due to its small size and low iso-electric point (pH 3.9). With a diameter of 0.025 μm (Antony *et al.* 2011), FRNA presented a substantial challenge to removal via size exclusion by the membrane (pore diameter generally larger than 0.04 μm) and was chosen to model similarly sized enteroviruses present in wastewater. The low iso-electric point (pH 3.9 (Michen *et al.* 2010)) relative to the typical operating pH of MBR (7-8) (Judd 2011) reduced the likelihood of adsorption of FRNA to the membrane, as above pH 3.9, the virus particle carries a net negative charge (Antony *et al.* 2011). Hence, FRNA is recommended as a potential virus indicator given, well-documented previous use and its conservative model properties.

When comparing removals of virus surrogates across MBR, somatic coliphage (SC) demonstrated higher resistance than FRNA bacteriophage (Appendix B). Indigenous SC can vary in size from 0.027 to 0.2 μm, as a result there is some question as to whether SC is suitably conservative as a challenge organism given that some species overlap significantly with the MBR pore size range of 0.04 to 0.4 μm. Nevertheless, data from 85 site visits yielded detection of SC in 58% of permeate samples, with a 5th percentile LRV of 2.5. In contrast, FRNA was detected less often at 28% of permeate samples and featured a resulting LRV of 2.9. The overall resistance of SC to MBR treatment would appear primarily due to the fact that it is poorly deactivated by the activated sludge. As a result, higher concentrations of SC reach the membrane surface, increasing the likelihood of passage into the permeate. FRNA has shown removal of up to 2.1 log within the activated sludge before reaching the membrane, making passage across the membrane and detection unlikely. Use of SC for validation appears to be current practice, as all previous validation studies used SC in place of FRNA.

3.2. Bacteria

Escherichia coli (EC) is recommended to represent bacterial pathogens, due to extensive historic use as fecal contamination indicators and as challenge

organisms for membrane systems. In addition, previous validation reports have used EC as a bacterial surrogate (Appendix C).

3.3. Protozoa

Clostridium perfringens (CP) is recommended as a surrogate for protozoa. Due to CP ability to form spores and resist hard environments, it has been used as a surrogate for cryptosporidium in disinfection studies (Venczel *et al.* 1997). Depending on the strain analysed, CP spore diameters range between 0.6 – 1 .0 µm (Orsburn *et al.* 2008). CP smaller size, relative to other protozoa (5 – 10 µm) (Antony *et al.* 2011), further supports its use as a conservative indicator in membrane challenge testing. Additionally, CP has been used as a challenge organism to represent protozoan removal in previous studies on MBR (Ottoson *et al.* 2006, Marti *et al.* 2011, van den Akker *et al.* 2014).

3.4. Knowledge Gaps and Outcomes from Current Work

Some concern over the lack of LRV correlation between CP and *Cryptosporidium* through activated sludge plants was raised. As only 3 LRV exist for *Cryptosporidium* in published literature (Ottoson *et al.* 2006) (Pettigrew *et al.* 2010), investigation of a correlation was not possible for MBR. Although more readily available than protozoa, pathogenic virus data has rarely been reported in conjunction with process indicators (Francy *et al.* 2012) (Kuo *et al.* 2010) (Sima *et al.* 2011). Of equal importance is that minimal or no process operating data has been supplied alongside pathogenic virus or protozoan removal.

In order to address this gap, SP1 submitted samples for analysis at Sydney Water Corporations West Ryde Laboratory for Cryptosporidium, Giardia and Viruses by cell culture (enterovirus, adenovirus and reovirus). On the same samples, UNSW has analysed SC, FRNA, EC and CP in addition to summarising relevant operational data. The full set of results will be available late July, used to supplement this section and make recommendation over the most suitable process indicators for MBRs.

4. Influencing factors

Influencing factors are operational elements that are expected to impact the treatment efficacy of pathogen reduction. From 33 published sources and 2 validation reports, 684, 597 and 26 LRV were analysed for indicators or pathogens from bacteria, virus and protozoan groups respectively. Of the 33 published sources, only 9 contained pathogen data, the balance included indicator organisms. The data were fit to normal distributions to describe LRV performance and variability through MBR. For the combination data sets, the 5th percentile LRV for virus, bacteria and protozoa were > 1.7, 3.5 and 3.5 respectively (Appendix B). Although mechanisms for pathogen removal by MBR are well known, no studies have provided satisfactory data to allow quantification and/or prioritization of important influencing factors. As a result, an extensive sampling program of over 180 site visits across 10 full scale MBRs was conducted in order to identify significant operating parameters.

Some trends were highlighted from the literature review conducted to investigate the current knowledge on MBR LRV (Appendix A). The main conclusions from these studies were:

- Membrane pore size between 0.04 and 0.4 μm does not appear to significantly affect virus rejection under steady state conditions.
- The overall significance of MLSS is unclear. For viruses, a higher MLSS concentration may present more adsorption sites.
- A longer SRT is expected to result in greater accumulation of organisms (rejected by size exclusion and resistant to biodegradation); hence, a higher biomass concentration challenging the membrane.
- F/M ratio may affect formation of a bio-fouling layer on the membrane that enhances virus rejection.
- Extreme pH changes may affect particle to particle and particle to sludge adsorption. Decrease in pH below the pathogen iso-electric point (IEP of around 3 – 5) may improve rejection. These extreme conditions do not fall in the typical operating envelope of MBR (pH 7 – 8).

- After long term operation, cleaning chemicals used to clean the membrane are expected to alter membrane material properties, but this effect on LRV has not been quantified in full scale systems.
- Long term chemical exposure may increase likelihood of integrity failure due to embrittlement of module materials and interfaces.

4.1. Bayesian Approach to Assess Factor Impact on LRV

MBRs feature a large number of, potentially interrelated, factors that could contribute to LRV. In order to identify significant factors, a Bayesian network was constructed, in conjunction with SP4 Multiple Barriers, to assess impact on indicator LRV. If a factor could be found significant, the effect of increasing or decreasing that factor could then be presented in terms of likely influence on LRV.

The MBR Bayesian network was trained on over 100 site visits worth of data. Node connections were informed through an iterative process, incorporating expert knowledge workshops and automated structure learning. Further specifics on construction of the current MBR Bayesian network can be found in Appendix D.

Indigenous influent indicator densities cannot be controlled; as a result, calculation of LRV can often relate in censored 'greater than' LRVs, where indicators are removed below the limit of detection. In order to circumvent this problem, the Bayesian network was interrogated to ascertain factors that, when changed, would increase the likelihood of higher activated sludge and permeate indicator densities. Given that influent densities were fixed in the model (according to the complete set of site visit data), factors that lead to high permeate indicator densities were equivalent to causes of low LRV. The generalized approach for use of a Bayesian network to identify effect and significance of influencing factors is illustrated in Figure 1.

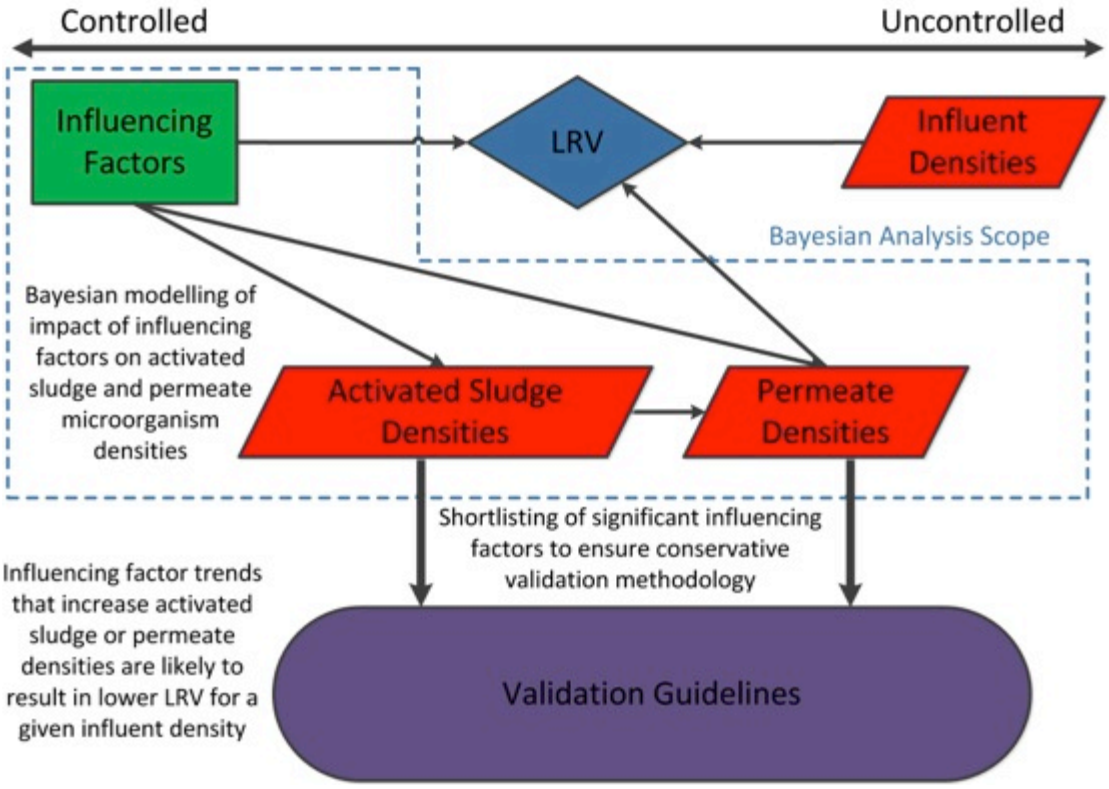

Figure 1 – A Bayesian approach to identification of significance and effect of influencing parameters on LRV and integration in the validation guidelines

Factors that could affect or relate to LRV were shortlisted in Table 2 from review of MBR design literature (Judd 2011), the Victorian validation guidelines (VDoH 2013) and previous validation reports (Appendices A and C). Factors were classified into types, according to when the parameters could be chosen, determined or changed. Factor types classification included:

- Design: Factors chosen at the design stage,
- Operational Control: Factors that could be altered at operation,
- Operational Measurement: Simple factors that are measured during operation but are controlled indirectly (eg can reduce TMP by lowering flux), and
- Water Quality/Measured Parameters: Factors that are measured but may not be possible to control directly.

Preliminary Bayesian analysis of MBR influencing factors suggested that operation under the following conditions resulted in a higher likelihood of a lower LRV:

- Low HRT
- High flux
- Low TMP
- High permeability
- Low MLSS
- High permeate turbidity and
- High dissolved oxygen in biomass

The inclusion of flux, TMP and permeability is under review, as these parameters are not wholly independent. The presence of high dissolved oxygen cannot be explained and is under further investigation (e.g. as it could be related to increased level of shear provided to the biomass flocs, potentially damaging them).

pH was investigated but did not yield significant changes in LRV. SRT influenced MLSS/MLVSS concentrations but did not influence the indicator densities in mixed liquor and permeate directly. DOC did not influence the indicator densities in mixed liquor and permeate directly, but changes in DOC in mixed liquor and permeate follow the same trends as changes in indicator densities in these samples, indicating that DOC is a potential surrogate for these indicators in mixed liquor and permeate. Similarly, CST did not directly influence indicator densities in mixed liquor and permeate. Analysis of membrane pore size suggested lower LRV at higher pore size. However, membrane pore size, material and configuration was not included in the major trends as regardless of the starting pore size, each membrane type will need to satisfy the same validation methodology. In addition, only 1 of 10 sites featured a higher range pore size (0.4 μm, flatsheet), as a result there maybe bias due to the sample set.

Temperature in mixed liquor was initially included for Bayesian analysis but was removed, in order to simplify the network, after not demonstrating significant

sensitivity to any other variables in the net. Temperature is still under investigation in further MBR Bayesian Network revisions. Membrane ageing resulted in an increased likelihood of higher virus LRV, but resulted in lower bacteria LRV. Accordingly, the membrane ageing relationship has been listed as uncertain and will be monitored on future revisions of the Bayesian network.

The full set of shortlisted factors including range of data analysed, significance and trends with LRV is summarised in Table 2.

Table 2 - Shortlisted influencing factors for MBR LRV and outcomes of the Bayesian modelling

Factor (units)	Factor Type[a]	Low, IQR, High[b]	Candidate in Bayes Net	Factor Range for Conservative LRV[c]	Comments
SRT (d)	C	12, 32 – 126, 147	Yes	No influence	
HRT (hr)	C	4, 17 – 39, 100	Yes	Low	
F/M ratio (gBOD/gMLVSS/d)	C/Q	0.02, 0.02 – 0.04, 0.06	No	N/A	Investigated by use of DOC as surrogate for organic matter concentrations
Flux (LMH)	C	0.4, 5 – 22, 37	Yes	High	
TMP (kPa)	M	0.4, 5 – 7, 50	Yes	Low	
Permeability (LMH/kPa)	M	0.1, 0.8 – 5.1 , 33	Yes	High	Quantifies current membrane fouling state
Membrane Type[d]	D	N/A	N/A	N/A	Same final performance testing requirement
Pore size (µm)	D	0.04 - 0.4	Yes	High	Most 0.04 µm.
Membrane Age (months)	M	1, 5 – 27, 217	Yes	Uncertain	To inform on revalidation conditions. Removal variability increased with age. Different effect for virus and bacteria.
Membrane Aeration	C	N/A	No	N/A	Used DO as a surrogate measured parameter
Membrane Area	D	N/A	No	N/A	
Chemical Cleaning[e]	C	N/A	No	N/A	Permeability as surrogate for fouling condition
Chemical Dosing[f]	C	N/A	No	N/A	Could be investigated qualitatively from site knowledge
MLSS/MLVSS (g/L)	Q	0.1, 3.4 – 12.9, 20	Yes	Low	Except for CP as low MLSS implies high wasting rate
CST (s)	Q	11, 22 – 44, 274	Yes	No influence	
COD (mg/L)	Q	268, 282 – 530, 2230	No	N/A	DOC as surrogate for organic matter concentrations
BOD (mg/L)	Q	60, 127 – 195, 353	No	N/A	DOC as surrogate for organic matter concentrations
DOC (mg/L)	Q	9, 61- 88, 182	Yes	No influence	
Ammonia (mg/L)	Q	7, 35 – 50, 95	No	N/A	Small data set
Turbidity (NTU)	M	0.01, 0.03 – 0.13, 3.7	Yes	High	Minimal high turbidities make correlation of turbidity poor
pH	C/M	3.8, 6.9 – 7.5, 9.0	Yes	No influence	
DO (mg/L)	M	0, 1.5 – 4.8, 8.3	Yes	High	
Temperature (°C)	M	16, 21 – 25, 30	No	N/A	Low sensitivity to findings

[a] D = Design, C = Operational Control , M = Operational measurement, Q = Water Quality/offline measured parameter
[b] IQR = Interquartile range, Low = lowest and High = highest, of parameters from SP1 Full Scale Site Sampling
[c] If factor range is high means that for high levels of that factor the likelihood of a low LRV increases. No correlation indicates no discernable trend for range tested
[d] Includes membrane material and membrane configuration
[e] Includes chemical type, cleaning duration and mode (eg CIP or CEB, backwash or manual cleaning).
[f] Includes pH adjustment, coagulant addition and possible feedwater BOD supplement.

5. Identification of Operational Monitoring Parameters

Due to the complex mechanisms of MBR, no single set of operating parameters has been identified which would demonstrate the removal performance in real time. Operational monitoring should target both confirmation of membrane integrity and activated sludge health performance.

Common membrane integrity monitoring techniques applicable to MBR were critically assessed, including turbidity, particle counting and pressure decay testing (PDT) (Appendix E). Turbidity and particle counting are applicable for online monitoring of membrane integrity for all configurations of MBR. However, the limit of detection of these techniques with regards to pathogen breakthrough remains unknown. PDT cannot be considered as an online monitoring technique, and is only applicable to certain configurations of MBR. Validation protocol of MBR, for which PDT is applicable, has been suggested to be enacted as for direct membrane filtration in VDoH 2013. As such, PDT will not be investigated further.

Activated sludge performance may be confirmed by monitoring of nutrient removal performance (ammonia and DOC); however the correlation with LRV is, at present, unknown.

LRV by the membrane alone are 2 to 5 times higher than by the activated sludge (van den Akker *et al.* 2014). As a result, priority should be given to monitoring membrane integrity over bioreactor performance.

6. Description of Validation Methodology

A validation study should be conducted representing the worst-case expected operational conditions to provide confidence that accredited LRV will be achieved during normal operation. Thorough risk assessment should be conducted covering site and design specific conditions. The validation study should challenge test the MBR, by measurement of influent and permeate microorganism densities, in order to calculate a $LRV_{C\text{-}test}$ representative of Viruses, Bacteria and Protozoa removal.

6.1. Validated operating envelope

A full set of influencing factors must be defined in order to set the boundaries on when the MBR is validated. Choice of influencing factors and boundaries should be supported by risk assessment. From preliminary Bayesian analysis, key operational parameters and conditions for a conservative LRV included operation at:

- Low HRT
- High flux
- Low TMP
- High permeability
- Low MLSS
- High dissolved oxygen in the bioreactor

As a result, the validation sampling program should be conducted at the lowest expected HRT, TMP, MLSS and highest expected flux, permeability and dissolved oxygen. For example, if a process were validated at 5 hr HRT then providing HRT was greater than 5 hr during operation, permeate could be produced with reasonable confidence that $LRV_{C\text{-}test}$ was being maintained or exceeded.

In addition to parameters listed as significant, it is recommended that other potential influencing factors, not limited to those shortlisted in Table 2, are documented during the validation study. Membrane parameters including supplier, model number, configuration, nominal pore size, membrane material,

planned chemical cleaning frequency and replacement schedule should be documented for each validation study. In addition, membranes installed during a validation study should be selected according to the MFGM (USEPA 2005), i.e.:

- The tested membranes are representative of the overall product variability. Ideally tested membranes will be from production lots close to the conservative end of quality control testing.
- At least five different membranes, from different manufactured lots are to be tested.

6.2. Pre-validation

Pre-validation, i.e. the use of a previous validation report, published literature and/or operational monitoring set as a proof of $LRV_{C\text{-}test}$, could be considered under some circumstances. To be eligible for pre-validation, it must be satisfactorily proven that the previous validation results apply to the new circumstance. Reasonable examples where pre-validation may apply include:

- A package plant MBR installed and operated under defined, previously validated conditions.
- A new plant constructed and operated according at the same conditions of previously validated sister plant.
- Extensive validation of a specific MBR product, with an appropriate operating envelope, from the supplier.

Figure 2 is included to illustrate pre-validation eligibility.

Figure 2 - Eligibility for pre-validation

7. Method to collect and analyse validation data

If a proponent is not eligible for pre-validation, sufficient data must be collected from onsite challenge testing. Challenge testing is defined here as the analysis of influent and permeate samples for target microorganisms in order to determine LRV. Target microorganisms may be indigenous, or specific species may be spiked where influent concentration is too low. In order to avoid interferences due to interactions between high concentrations of target microorganisms, it is recommended that no more than 3.16×10^6 multiplied by permeate LOD, of the spiked target microorganism, is used as the feed water challenge concentration in accordance with the MFGM (USEPA 2005). Onsite challenge testing should be conducted according to the following steps:

1. The MBR should be equilibrated and operated at the worst-case boundaries of the operational envelope for the validation period (Section 6.1),
2. Process indicators, representative of the target pathogen for validation, should be analysed on paired influent and permeate samples,
3. Operational data, especially key influencing factors, must be documented for each sample set,
4. The sample frequency and duration should be sufficient to permit robust statistical analysis of LRV data.

7.1. $LRV_{C\text{-}test}$

$LRV_{C\text{-}test}$ can be calculated with Equation 1

$$LRV_{C-test} = \log_{10} \left| \frac{C_{In}}{C_{Perm}} \right| \qquad \text{Equation 1}$$

Where C_{In} and C_{Perm} are paired daily process indicator densities of influent and permeate samples, respectively. Where a process indicator is not detected in the permeate, the method detection limit should be used as the value of C_{Perm} and the resulting $LRV_{C\text{-}test}$ expressed as "> LRV".

7.2. Sampling Location and Time

Grab samples of influent to the MBR and permeate, before any additional disinfection process should be taken. Grab, as opposed to composite, samples were previously suggested to capture more process variability at a full-scale MBR (van den Akker *et al.* 2012). The densities of relevant process indicators should be evaluated for each target pathogen under investigation (Table 1). Sufficient replications of samples and/or analysis for any paired set of influent and permeate to justify adequate QA/QC. Triplicate sampling is generally recommended.

In order to ensure a conservative LRV, permeate samples should be taken as soon as reasonably practical after normally occurring backwash or relaxation events. In order to ensure the highest permeability of the operating envelope during the validation period, it is recommended to validate the membranes when near new or after chemically cleaning. If revalidation or validation of a used, fouled membrane is to be conducted it must first be cleaned (to the permeability of the desired operating envelope). As a result, any integrity failures will be exposed and results of challenge testing will represent the performance of the membrane in its current state. Sodium hypochlorite (NaOCl) is a common membrane cleaning chemical as well as a strong disinfectant. Adequate time should be allowed for NaOCl or other cleaning chemicals to dissipate from permeate pipework before sampling. Additional measurements to ensure no oxidiant residual be present should be taken and documented before samples for process indicators.

7.3. Sampling Period and Frequency

An adequate sampling period and frequency of sampling should be justified with the aim of generating a statistically valid set of results that can allow enough time for true process variability to be observed.

Previous validation studies have enacted a sample regime varying from weekly analysis for 14 weeks, up to 3 times per week for 10 weeks. In order to obtain initial estimates of descriptive statistics, no less than 7 and ideally more than 15 samples were recommended in a recent NSW office of water guidance document for water recycling (NSW_OFW 2015). When evaluating MF and UF membranes, the MFGM recommends that the 5th percentile LRV from challenge testing can be adopted over the minimum LRV when the number of membranes tested is great than 20 (USEPA 2005). VDoH 2013 recommends sampling 6 samples over 3 different fouling regimes (low, medium and high). In addition, the sampling should take place over seasonal variation or, at the worst season for operational performance if known. Temperature variation from 16 to 30 °C did not appear as a significant influencing factor of LRV (Table 2). High flow events due to seasonal rainfall may force an MBR to operate at higher fluxes and lower HRTs. Provided the MBR has sufficient operating envelope in the original validation plan, seasonal effects of temperature (16 – 30 °C) and rainfall should not be a basis for extending the validation period.

In light of previous work, a sampling program is recommended with no more than 2 samples per week, on non-concurrent days, over a minimum of 3 months to at least 15 samples are proposed. With greater than 20 samples, $LRV_{C\text{-}test}$ can adopt the 5th percentile LRV, otherwise $LRV_{C\text{-}test}$ is equal to the lowest paired LRV from the challenge period.

8. Critical Limit Determination

A philosophy was adopted where validation testing should be conducted during a worst-case operational envelope based on significant influencing factors, allowing determination of LRV_{Ctest}. In order to ensure a process continually

achieves the required performance, an online monitoring technique must be correlated with LRV. The resolution of the chosen online monitoring technique will determine a maximum LRV able to be demonstrated. Upon correlation of LRV with online monitoring response, a critical control limit (CCL) can be chosen. The LRV corresponding to the chosen CCL (LRV_{CCL}) can be used as a measure of removal performance that can be continually be monitored. A monitoring technique is suitable provided, a reasonable explanation of how the technique monitors a critical control point can be made and a correlation of LRV with monitoring response can be shown.

Previous studies that measured the contribution of activated sludge as well as the membrane on pathogen retention in MBRs demonstrated that a majority of removal occurs across the membrane (van den Akker *et al.* 2014, Chaudhry *et al.* 2015). Viruses, typically smaller than the membrane pore size, are predominantly attached to suspended solids within the activated sludge of an MBR (Oota *et al.* 2005, Sima *et al.* 2011, Simmons *et al.* 2011). Turbidity measures 90° light scatter and provides a response proportional to the amount of particulates in a solution. The activated sludge compartment of an MBR contains between 3 – 14 g/L of suspended solids. If a breach in the membrane were to occur, transfer of suspended solids into the permeate at concentrations detectable by turbidity is likely. Given that smaller virus particles are generally attached to suspended solids, turbidity may show some sensitivity to virus LRV in MBR.

As there is reasonable evidence to suggest that turbidity should indicate membrane integrity in MBR, the rest of this section suggests a method on how turbidity, if chosen, may be correlated with LRV. The VDoH guidelines have proposed a method for correlation of turbidity with LRV. Previous validation reports have set a CCL for turbidity at 0.2 – 0.5 NTU, based on research from membrane suppliers. Generally, when CCLs are exceeded, a timeframe specified to reduce chance of false positive is considered before corrective actions are implemented. Control strategies have included bypass to head of works or waste, or plant shutdown. The CCL and corrective actions must be documented as part

of the recycled water quality management plan (Similar examples have been reported in Appendix C).

An example approach to correlate turbidity, measured in MBR permeate, with LRV is presented below. A minimum of 6 paired samples over different permeate turbidities should be taken of influent and permeate with LRVs calculated with Equation 1. Before attempting to correlate turbidity and LRV, ensure that turbidity meters are cleaned, calibrated and installed as per manufacturer instructions. In order to generate higher turbidities, two approaches are plausible:

- Approach 1: Use a dosing pump to bypass mixed liquor into the permeate line at increasing dosages while noting the bypass ratio. Begin at the lowest ratio and finish at the highest ratio. Correlate the bypass flow with an expected membrane damage rate, based on a flow dilution model. Express LRV and turbidity results as illustrated in Figure 3.
- Approach 2: Sequentially damage membranes by systematically cutting fibres or slicing sheets in order to allow bypass of MLSS particles into the permeate. Record turbidity and damage rate. It may be necessary to backflush membranes with air or liquid to avoid turbidity recovery due to plugging of membrane defects with activated sludge flocs.

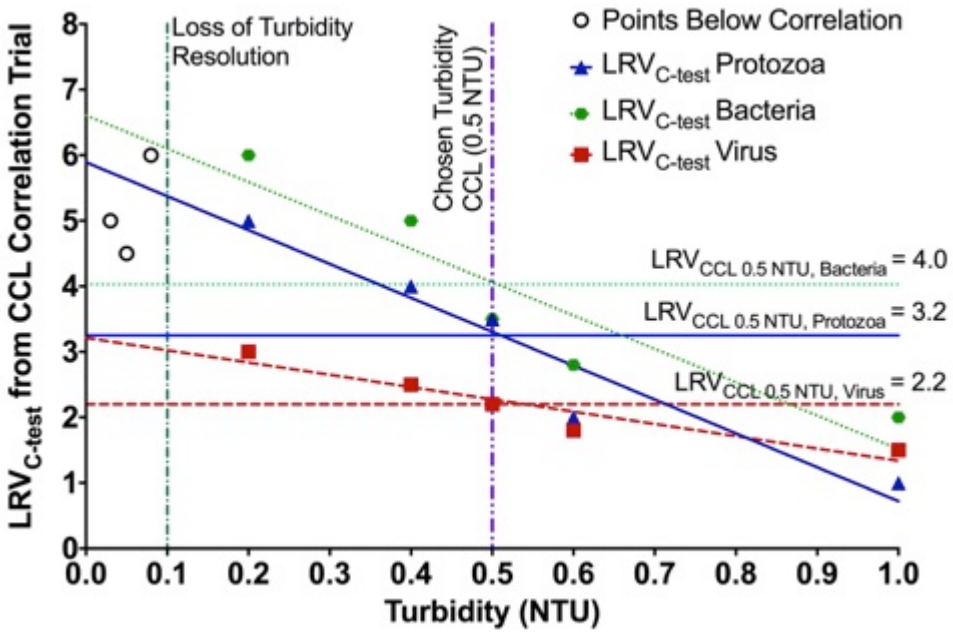

Figure 3 - Illustration of CCL determination for turbidity. Not real data

After following either Approach 1 or 2, data should be available to allow creation of a plot similar to Figure 3. A CCL for turbidity can then be chosen and a corresponding LRV_{CCL} selected at the point where the $LRV_{C\text{-}test}$ correlation meets the chosen turbidity. It is likely that significant loss of resolution will occur at low turbidities. Sampling should only take place where there is a certain measurable change. Before designing a sampling program, it may be worth assessing historical turbidity data to ascertain the normal baseline. The CCL must be chosen within the range of correlated values and greater than the value where loss of resolution occurs. The sampling program should be conducted under the same conservative conditions identified in the validation methodology. Turbidity correlation should be performed at the lowest MLSS concentration in the operating envelope.

9. Determination of Accredited Log Removal Value

Consistent with the approach outlined in the VDoH 2013, The log removal credit (LRC), i.e. the LRV that may be attributed to a treatment process unit is the lowest value of either the:

- validated $LRV_{C\text{-}test}$ demonstrated during challenge testing, or
- LRV_{CCL} that can be verified at the chosen critical control point by the operational monitoring technique used to measure the efficacy of the treatment process unit to reduce the target pathogen (i.e. the sensitivity of the operational monitoring technique).

Figure 4 is included below to clarify the decision making process.

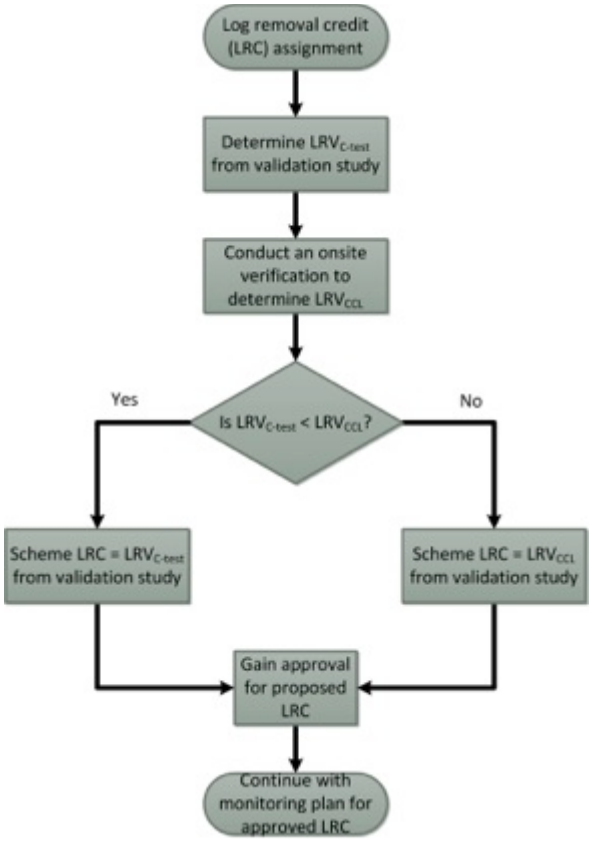

Figure 4 - Assignment of validated LRV

In most cases, the LRV attributed to a treatment process unit will be limited by the sensitivity of the chosen operational monitoring technique and the stringency of the CCL.

10. Triggers for Revalidation

Revalidation conditions were summarised from VDoH 2013, previous validation reports and existing recycled water quality management plans. They include:

- Changes to the treatment process that would exceed the validated operating envelope such as:
    - Design modifications,
    - Control philosophy or operating parameters,
    - Membrane replacement with a different model to the one validated,
- Changes to the intended use,
- Changes in legislation,
- Continual breaches of CCL.

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Appendix B:

Consideration for default LRV in MBR

Executive Summary

A tiered approach is proposed in order to address concerns for smaller schemes over the cost associated with validation. At the lowest tier, minimal validation activities would be required and a conservative default log removal value (LRV) could be adopted, providing operation meets standard criteria and would provide a basis for unit operation selection during early scheme design phases.

In order to establish a default LRV for MBR, a large body of literature and full scale site data was considered. In addition, samples were taken for determination of virus and protozoan pathogens. From the data collected, either from full scale sites or literature, a worst case LRV was determined.

The 5th percentile for the combined data set on virus LRV extracted from literature was 1.7, identical to the value determined for somatic coliphages from full scale site data, but more conservative than the > 3.3 observed as the minimum enterovirus LRV. 5th percentile protozoan LRV indicated by *clostridium perfringens* was 2.5, lower than the minimum *cryptosporidium* LRV of >3.1. *E.coli* LRV from full scale site data was 4.8 at the 5th percentile. For all bacteria LRV isolated from literature, the 5th percentile was 4.6. Given that a maximum LRV of 4.0 per unit operation is adopted to encourage multiple barriers in a reuse scheme, bacteria removal does not appear to be a concern for MBR. When only membranes with an installed pore size of < 0.1 µm were considered 5th percentiles for somatic coliphages, FRNA bacteriophages, *E. coli* and *clostridium perfringens* increased to 2.0, 3.5, 5.5 and 5.2, respectively.

A further conservative step was introduced whereby the LRV was rounded down to the nearest half log.

An operational envelope corresponding to the LRVs observed during full scale site visits was established (Table 8 and 9). Turbidity was the monitoring technology employed at a majority of sites to ensure permeate quality and did not exceed 0.4 NTU at the 95th percentile. Provided a MBR is operated within the range of parameters assessed in Table 8, the following default LRV are proposed for MBR:

Virus: 1.5

Bacteria: 4.0

Protozoa: 2.0

If further limitations are imposed, such as installing membranes with a pore size < 0.1 µm, not exceeding a flux of 30 L/m^2/h or permeate turbidity at the 95th percentile of 0.3 NTU then the following default values may be valid:

Virus: 1.5

Bacteria: 4.0

Protozoa: 4.0

Table of Contents

1 Introduction

Concern has been raised over the significant cost of validation activities prohibiting smaller schemes from being able to produce recycled water, even if a conservative flow sheet is installed. One approach to minimise cost would be a tiered approach to validation. Tier 1 would be to allocate a conservative default log removal value (LRV) and associated operating envelope to technologies. Tier 2 and higher may achieve greater accredited LRVs but would necessitate further validation activities, with the expectation of higher cost.

A default or indicative LRV would also be beneficial, as it would provide a basis for unit operation selection during early scheme design phases.

This report aims to suggest default LRVs that could be adopted for virus, bacteria and protozoa by a membrane bioreactor (MBR). In order to achieve this, MBR literature was extensively searched and LRV data extracted. In addition, over 180 site sampling visits were conducted to MBRs in NSW, VIC and SA.

2 Method

2.1 LRV collation from literature

Virus, Bacteria and Protozoa indicator and pathogen LRV were extracted from 33 published literature sources reporting on MBRs. LRV values were either calculated from concentration data or tabulated values. If presented graphically, values were interpolated from figures using PlotDigitiser (Ver 2.6.3, http://plotdigitizer.sourceforge.net). The resulting database contained sufficient data to calculate 590, 670 and 20 LRVs viruses, bacteria and protozoa, respectively (Appendix A- MBR LRV from Literature).

References to papers where data was extracted are listed below. (van den Akker *et al.* , Chiemcharisri *et al.* 1992, Dowd *et al.* 1998, Gander *et al.* 2000, Ueda *et al.* 2000, Chang *et al.* 2001, Gantzer *et al.* 2001, Mooijman *et al.* 2001, Severn 2003, Oota *et al.* 2005, Shang *et al.* 2005, Xiang *et al.* 2005, Friedler *et al.* 2006, Hirani *et al.* 2006, Lv *et al.* 2006, Ottoson *et al.* 2006, Zheng *et al.* 2006, DeCarolis *et al.* 2007, Silva *et al.* 2007, Tam *et al.* 2007, Zhang *et al.* 2007, Hirani *et al.* 2010, Kuo *et al.* 2010, Michen *et al.* 2010, Nishimori *et al.* 2010, Pettigrew *et al.* 2010, Wu *et al.* 2010, Zanetti *et al.* 2010, Marti *et al.* 2011, Mosqueda-Jimenez *et al.* 2011, Sima *et al.* 2011, Simmons *et al.* 2011, Francy *et al.* 2012, Hirani *et al.* 2012, Keskes *et al.* 2012, Trinh *et al.* 2012, van den Akker *et al.* 2012, De Luca *et al.* 2013, Hirani *et al.* 2013)

A majority of the references reporting LRV did not have sufficient detail on associated operating conditions. As a result a full scale MBR sampling operating consisting of over 180 total visits was conducted by SP1. On each visit operating data as well as indicator LRV was collected as a paired set.

2.2 Site selection and sampling

A summary of the sites, number of sampling visits and usefulness of particular MBRs with respect to guideline development was included in Table 1

Table 1 - Summary of site selection and number of visits conducted by SP1

Sites	**# of visits**	**Site specific details**
Site 1	17	• Flat sheet (Kubota RW515), large pore size (0.4 µm), high flux, 4 independent permeate trains. Omitted in revised default values for pore size < 0.1 µm.
Site 2	9	• Flat sheet (Biocell, 0.04 µm), gravity driven, 7 year old • No further visits due to difficulty obtaining monitoring data
Site 3	30	• Hollow fibre (Zenon ZW 500D, 0.04 µm), moderate flux, intermediate SRT & HRT, < 1 year old, 2 independent permeate trains • Sampling for Cryptosporidium and Viruses • Online monitoring trial
Site 4	17	• Hollow fibre (Zenon ZW 500D, 0.04 µm), moderate flux, intermediate SRT and HRT, 6 independent permeate trains, 2 years old • Chemical Cleaning study
Site 5	3	• Hollow fibre (Evoqua B40, 0.04 µm) • No further visits due to difficulty obtaining monitoring data
Site 6	3	• Flat sheet (Biocell, 0.04 µm), gravity driven, < 1 year old • No further visits due to difficulty obtaining monitoring data
Site 7	7	• Hollow Fibre (Koch Puron, 0.1 µm)
Site 8	1	• Ceased sampling due to lack of available operating data
Site 9	61	• High/constant flux, short HRT, short SRT, hollow fibre (Evoqua B10, replaced with B40, 0.04 µm) • Sampling for Cryptosporidium and Viruses • 10 year old membranes sampled before and after replacement • currently running with new generation
Site 10	40	• 3 year old, hollow fibre (Zenon ZW 500D, 0.04 µm), long HRT, long SRT, high MLSS, low flux • Online monitoring trial • Chemical cleaning trian
Site 11	1	• Hollow fibre (Evoqua B40, 0.04 µm) • No further visits due to difficulty obtaining monitoring data
Total Visits		181

At each site, indicator microorganisms, bulk parameters and operational data was collected and analysed. Table 2 contains a summary of parameters and analysis undertaken for each site visit.

Table 2 - Sampling and data from each site visit

Parameter Type	**Test**	**Sample Location**
Virus Indicators	FRNA Bacteriophage	Influent, Activated Sludge and Permeate
	Somatic Coliphage	
Bacteria Indicators	E. Coli	
	Total Coliforms	
Protozoa Indicator	Clostridium Perfringens	
Bulk Parameters	Dissolved Organic Carbon	
	Fluorescence Excitation Emission Matrix Spectra	
	pH	
	MLSS/VSS	Activated Sludge
	Capillary Suction Time	
	Dissolved Oxygen	*SCADA
	Turbidity	Permeate
Operating Data	Trans-membrane Pressure	SCADA
	Flux	SCADA
	SRT	SCADA
	HRT	SCADA

The master table containing all LRV data and operating parameters was included in (Appendix B – MBR Data Sheet). Where possible, additional information was extracted from existing validation reports. Not all corresponding operating

parameters and site information was available from validation reports. However, further validation performance data from site commissioning was added for Sites 1, 3, 10 and an additional Site 12, not tested by UNSW.

2.3 Selection and analysis of indicators

Direct measurement of pathogenic species in wastewater is often not feasible due to low and highly variable concentrations, and complex analysis procedures (Antony *et al.* 2011). As a result, indicator organisms are often chosen as surrogates for pathogens. A suitable indicator organism should be selected such that it displays correlated or more conservative removal than the target pathogen (VDoH 2013).

FRNA bacteriophage (FRNA) has been investigated in several previous studies of log removal in MBRs (Severn 2003, Ottoson *et al.* 2006, Hirani *et al.* 2010, Pettigrew *et al.* 2010, Francy *et al.* 2012, Hirani *et al.* 2012, van den Akker *et al.* 2014). FRNA was selected as an indicator of virus removal performance due to its small size (0.025 µm) (Antony *et al.* 2011) and low iso-electric point (IEP) (pH 3.9) (Michen *et al.* 2010). With a diameter of 0.025 µm, FRNA presents a substantial challenge to removal via size exclusion by the membrane (pore diameter generally larger than 0.04 µm) and was chosen to model similarly sized pathogenic viruses present in wastewater such as, enterovirus (0.022 – 0.030 µm, IEP 4.0 – 6.4) and hepatitis A (0.027 – 0.028 µm, IEP 2.8) (Xagoraraki *et al.* 2014). A low IEP (pH 3.9) relative to the typical operating pH of MBR (7-8) (Judd 2011) reduced the likelihood of adsorption of FRNA to the membrane, as above pH 3.9 the virus particle carries a net negative charge (Antony *et al.* 2011).

Indigenous somatic coliphages (SC), can range in size from 0.027 – 0.1 µm (Wu *et al.* 2010). SC has been reported to exist at higher concentrations in the activated sludge when compared to the influent to an MBR (Marti *et al.* 2011, Mosqueda-Jimenez *et al.* 2011). Previously, the higher density of SC has been explained by the ability to infect a variety of E. *coli* hosts and replicate in the environment (Grabow 2001). The potential for replication has been disputed, as in most cases, there should not have been sufficient concentration SC hosts to allow replication

(Jofre 2009). Consequently, an explanation for whether accumulation of SC within the activated sludge of MBR is due to, growth or resistance to biological predation, is still unavailable. Regardless of the reason for accumulation of SC, the LRV demonstrated is of a conservative nature, due to the fact the membrane is being challenged at a higher concentration, than that of the feed water. Hence, FRNA and SC were both evaluated as virus indicators given their well-documented previous use and conservative model properties.

FRNA were quantified using the double agar layer (DAL) technique according to previously published methods (Noble *et al.* 2004), using E. coli F-amp (ATCC #700891) as the host and MS2 bacteriophage (ATCC #15597-B1) as the positive control. SC were also analysed by the DAL technique with E. coli CN-13 (ATCC #700609) as the host and Phi X174 (ATCC # 13706-B1) as the positive control.

Escherichia coli (EC) was chosen to represent bacterial pathogens, due to their extensive historic use as fecal contamination indicators and as challenge organisms for membrane systems. Brilliance agar (Oxoid CM1046) was used to enumerate EC after inoculation and incubation at 37 °C for 24 h. EC was obtained by only counting the blue colonies formed on the plate.

Clostridium perfringens (CP) was selected as a surrogate for protozoa. Due to CP ability to form spores and withstand harsh environments, it has been used as a surrogate for *Cryptosporidium parvum* in disinfection studies (Venczel *et al.* 1997). Depending on the strain analysed, CP spore diameters range between 0.6 – 1 .0 µm (Orsburn *et al.* 2008). CP smaller size, relative to other protozoa (5 – 10 µm) (Antony *et al.* 2011), further supports its use as a conservative indicator in membrane challenge testing. Additionally, CP has been used as a challenge organism to represent protozoan removal in previous studies on MBR (Ottoson *et al.* 2006, Marti *et al.* 2011, van den Akker *et al.* 2014). CP was enumerated using the tryptose sulphite cycloserine agar for CP (Oxoid CM0587), and incubated anaerobically at 37 °C for 24 hr.

CP and EC measured within the permeate were quantified using membrane filtration (Method 9215D, (APHA 1992)), whereby a desired volume of sample (typically 5, 50 and 100 mL) was filtered through a 47 mm diameter, 0.45 µm gridded filter membrane (Millipore, S-Pak, type HA). The filter membrane was then transferred onto the surface of a plate of selective agar. SC and FRNA permeate samples were plated as 10mL over 4 plates of appropriate bottom agar layer. Data was reported in colony forming units (CFU) for CP and EC and plaque forming units (PFU) for phage per 100 mL volume of sample. For SC and FRNA, LOD was 10 PFU per 100 mL. For CP, and EC, LOD was 1 CFU per 100 mL.

2.4 Analysis of pathogens

Samples were taken for analysis of *giardia, cryptosporidium*, enteroviruses, adenoviruses and reoviruses, on 8 occasions across two sites. Samples were transported to Sydney Water Corporations West Ryde Laboratories (SWC) on the day of sampling. For each of the two sites, 2 x 1 L samples of influent and activated sludge were taken on 4 occasions and 2 x 100L of permeate on three occasions for analysis of protozoa and viruses.

2.4.1 Analysis and reporting of protozoa LRVs

SWC utilised an in-house NATA accredited method for analysis of *giardia* and *cryptosporidium* based on (USEPA 2005). DAPI staining was used for confirmation of cysts and oocysts. Prior to analysis, permeate samples were concentrated, by SWC, using ultrafiltration. Results reported in this document are corrected for recovery and refer to DAPI positive (DAPI+) cyst or oocyst counts. *Giardia* and *cryptosporidium* were not detected in any of the six 100L permeate samples taken. LRV was calculated for same day samples, using the limit of detection (LOD = 1 DAPI+ Org/100L)) adjusted for the worst recovery. As a result, reported LRVs are conservative.

2.4.2 Analysis and reporting of virus LRVs

SWC utilised an in-house NATA accredited tissue culture method for analysis of enteroviruses, adenoviruses and reoviruses to yield most probable number (MPN). Influent and activated sludge samples were concentrated via PEG precipitation prior to analysis. 100L permeate samples were concentrated using

ultrafiltration. Simillar methods to those used by SWC have been reported previously (Keegan *et al.* 2012). Adenovirus, enteroviruses and reoviruses were not detected in any of the six 100L permeate samples taken. LRV was calculated for same day samples, using the limit of detection (LOD = 1 MPN/100L for enteroviruses and adenoviruses and 3.6 MPN/100L for reoviruses)). As a result, reported LRVs are conservative.

2.5 Probability density functions of LRV data

Probability density functions representing LRV were fit to data obtained from literature, site visits and validation reports. Depending on data suitability, one of three different fitting methods had to be used. Method 1 involved fitting lognormal probability density functions (PDF) to influent and permeate concentrations and then using Monte Carlo simulation for calculation of an LRV distribution. Method 1 could only be used if both influent and permeate concentrations were reported and greater than three different values existed above the LOD for each set. Method 2 used paired LRVs and fit a normal PDF to the lower LRVs that were not censored, extrapolating through 'greater than' censored LRVs calculated using the permeate LOD. Method 2 could only be used if there were at least 3 non-censored LRVs as the lowest values. Method 3 fit a normal distribution to all paired LRVs and assumed censored values, calculated with the permeate detection limit, were the true value. As a result, Method 3 was the most conservative and was able to be used on all data sets. PDF fitting and Monte Carlo simulation was performed with Pallisade @risk ver. 6. Further detail on fitting methods can be found in Appendix C – Data fitting methods.

2.5.1 Data reporting

Data regarding pathogenic viruses, indicator viruses, bacteria and protozoa were grouped into 4 tables. For pathogen groups of interest, the normal distribution fit parameters mean (μ) and standard deviation (σ), as well as the 5^{th} and 95^{th} percentile LRV of the model and the number of LRV (or concentration) data input were included in Tables 3 – 6. For fitting Methods 2 and 3, the ratio of LRVs that were detected in the permeate (d) over the total number of LRVs (n)

was reported. For fitting method 1 the d/n ratio was represented for as n/d F, n/d ML and n/d P for feed, mixed liquor and permeate concentrations respectively. Specific references containing the source data for normal distribution models were summarized in Table 7.

In cases where fitting Method 2 was not applicable, it was due to the presence of non-detects within at least the lowest 3 LRV reported. Where Method 1 was not applicable, the data has been combined across different sites or sources, or no concentration data was presented, only LRV. Method 3 was only considered non applicable (N/A) when all permeate were detected, hence fitting Method 3 was identical to Method 2. Where multiple sources have been used the number in brackets represents the number of independent data sources e.g. All Sources (3) was compiled from 3 different sources.

3 Results

3.1 LRV from literature

The data presented in Tables 3 – 6 were summarised as mean and standard deviation to permit quantitative microbial risk assessment (QMRA) of MBR processes either, deterministically, or probabilistically. In this format, both the LRV value and variability are expressed. 5th and 95th percentiles are also included. The 5th percentile may be used as an appropriately conservative starting point for default LRV.

Table 3 - Normal distribution parameters for MBR pathogenic virus LRV data

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
Adenovirus (species not specified)	All Sources (4)	N/A					N/A					3.37	1.14	1.49	5.25	16/24
	Source 1	3.87	1.36	1.64	6.08	11/11 F 5/11P	3.39	0.77	2.13	4.66	5/11	3.71	0.97	2.11	5.30	5/11
	Source 2	2.77	0.92	1.27	4.29	8/8 F, ML & P	2.74	0.34	2.18	3.30	8/8	N/A				
Adenovirus species A	Source 2	1.98	1.16	0.05	3.88	6/8 F 7/8 ML 8/8 P	2.33	0.33	1.79	2.87	6/6	N/A				
Adenovirus species C	Source 2	2.22	0.41	1.56	2.90	8/8 F, ML & P	2.23	0.31	1.73	2.73	8/8	N/A				
Adenovirus species F	Source 2	3.83	1.62	1.13	6.49	7/8 F 8/8 ML 8/8 P	3.47	0.71	2.30	4.64	4/7	3.41	0.64	2.35	4.47	4/7
Enteric viruses (by infectivity)	Source 1	N/A					N/A					2.55	0.48	1.76	3.33	0/8
Enterovirus	All Sources (5)	N/A					N/A					3.52	1.36	1.28	5.75	5/16
Norovirus (genogroup not specified)	All Sources (2)	N/A					N/A					4.45	1.92	1.23	7.61	8/15
Norovirus GI	All Sources (3)	N/A					N/A					3.33	1.65	0.62	6.05	12/30
	Source 3	4.28	1.61	1.61	6.90	14/14 F 13/14 ML 7/14 P	N/A					4.31	1.15	2.42	6.20	7/14
Norovirus GII	All Sources (2)	N/A					N/A					4.51	1.27	2.42	6.60	4/23
	Source 3	4.57	1.88	1.50	7.65	13/15F 15/15ML 4/15 P	N/A					4.49	1.84	1.48	7.51	4/15
Norovirus GIV	Source 3	N/A					N/A					3.22	0.64	2.18	4.27	0/6
Sapovirus	Source 3	N/A					N/A					2.62	0.76	1.36	3.87	1/16

Table 4 - Normal distribution parameters for MBR indicator virus LRV data

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
Bacteroides fragilis phage	All Sources (3)	N/A					N/A					3.66	0.60	2.67	4.64	0/9
FRNA bacteriophage	All Sources (8)	N/A					4.93	1.23	2.91	6.95	110/130	4.58	0.97	2.99	6.17	110/130
	Source 4, FS 5 Year Study	5.93	1.61	3.34	8.61	77/77 F 12/77 P	N/A					4.61	0.92	3.09	6.12	12/77
	Source 5, HF At 5 Years	5.71	0.92	4.20	7.22	6/6 F 3/6 P	N/A					5.29	0.53	4.42	6.16	3/6 LRV
MS2 bacteriophage	Source 6	N/A					N/A					4.14	1.45	1.75	6.52	10/48
Qβ bacteriophage	Source 7 (0.03 – 0.1 μm)	5.50	1.07	3.75	7.26	9/9 F 9/9 P	5.57	1.31	3.42	7.72	9/9	N/A				
Somatic coliphage	All Sources (9)	N/A					3.58	0.85	2.18	4.99	84/139	3.43	0.77	2.17	4.69	84/139
	Flat Sheet 0.4 μm (6)	N/A					3.33	0.54	2.45	4.21	27/28	3.46	0.77	2.20	4.72	27/28
	Hollow Fibre (0.04 μm) (3)	N/A					3.58	0.82	2.23	4.94	33/84	3.65	0.61	2.65	4.66	33/84
T4 coliphage	Sources (8, 9) 0.22 μm	6.13	1.77	3.23	9.03	31/31 F 21/31 P	7.44	3.18	2.20	12.67	21/31	5.99	0.62	4.97	7.02	21/31
	Sources (8, 9) 0.10 μm	5.87	1.16	3.98	7.79	17/17 F 7/17 P	5.97	1.43	3.62	8.32	7/17	5.83	1.03	4.13	7.53	7/17
Total coliphage	All Sources (2)	N/A					4.42	0.80	3.11	5.73	15/41	4.39	0.36	3.80	4.97	15/41
	Source 10 0.04 μm HF	N/A					4.53	0.13	4.32	4.73	7/12	4.59	0.24	4.20	4.98	7/12
	Source 10 0.4 μm HF	4.33	1.48	1.91	6.76	13/13 F 7/13 P	3.81	0.61	2.80	4.81	7/13	4.05	0.83	2.69	5.41	7/13
All Virus	Indicators & Pathogens	N/A					6.65	2.56	2.43	10.85	334/597	3.95	1.35	1.73	6.16	334/597

Table 5 - Normal distribution parameters for MBR bacteria LRV data

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
E.coli	All Sources (12)	N/A					N/A					5.87	0.71	4.71	7.04	40/95
	Source 11 incl. readings around cleaning	5.12	0.97	3.51	6.72	23/23 F 15/15 ML 15/18 P	N/A					N/A				
	Source 5 MBR at 5 years	5.78	0.37	5.17	6.38	6/6 F 6/6 P	5.71	0.26	5.28	6.14	6/6	N/A				
Enterococci	All Sources (5)	N/A					5.83	0.78	4.55	7.12	12/17	5.81	0.72	4.62	6.99	12/17
	Source 2 FS 0.4 μm	6.29	0.89	4.82	7.75	11/11 F 7/11 P	6.30	1.06	4.56	8.04	7/11	6.08	0.70	4.93	7.23	7/11
Faecal Coliforms	All Sources (10)	N/A					5.49	0.69	4.35	6.62	196/361	6.01	0.72	4.83	7.19	196/361
	Source 4 FS 5 years operation	5.89	0.50	5.05	6.71	143/143F 113/143P	6.03	0.67	4.93	7.13	113/143	5.97	0.62	4.95	6.98	113/143
	Source 4 FS 2.5 years operation	5.90	0.68	4.77	7.02	77/77 F 50/77 P	6.11	0.97	4.50	7.71	50/77	5.98	0.68	4.86	7.10	50/77
	Source 6	N/A					N/A					5.89	0.57	4.96	6.83	10/42
Total Coliforms	All Sources (11)	N/A					6.92	1.96	3.69	10.1	107/261	6.17	1.21	4.17	8.16	107/261
	Source 10 HF 0.08 μm	5.21	1.03	3.50	6.90	28/28 F 28/28 P	5.21	1.06	3.46	6.95	28/28	N/A				
	Source 10 HF 0.40 μm	6.62	0.64	5.57	7.67	13/13 F 9/13 P	6.59	0.45	5.86	7.33	9/13	6.61	0.53	5.74	7.48	9/13
	Source 11 incl. readings around cleaning	5.62	0.76	4.38	6.87	24/24 F 17/17 ML 13/14 P	N/A					N/A				
All Bacteria	Indicators and Pathogens	N/A					8.09	2.12	4.60	11.6	355/684	6.02	0.84	4.64	7.41	355/684

Table 6 - Normal distribution parameters for MBR indicator protozoa LRV data

Indicator	Parameter	Method 1					Method 2					Method 3				
		μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n	μ	σ	5th	95th	d/n
Clostridium Perfringens	All Sources (5)	N/A					N/A					4.61	0.41	3.95	5.28	20/21
	Sources 11,13,14 incl. readings around cleaning 0.1 - 0.2 µm HF	N/A					4.63	0.45	3.88	5.37	10/10	N/A				
	Source 12 FS 0.4 µm Pilot	N/A					4.65	0.34	4.10	5.21	10/10	N/A				
	Source 11 incl. readings around cleaning	5.16	0.59	4.20	6.11	20/20 F 17/17 ML 14/14 P	N/A					N/A				
All Protozoa	Indicators and Pathogens (mostly clostridia)	N/A					N/A					4.49	0.60	3.50	5.48	21/26

Table 7 - Specific references for normal distribution data

Source Number	Reference
1	Francy, D. S., E. A. Stelzer, R. N. Bushon, A. M. G. Brady, A. G. Williston, K. R. Riddell, M. A. Borchardt, S. K. Spencer and T. M. Gellner (2012). "Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters." Water Research 46(13): 4164-4178.
2	Kuo, D. H., F. J. Simmons, S. Blair, E. Hart, J. B. Rose and I. Xagoraraki (2010). "Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater." Water Res 44(5): 1520-1530.
3	Sima, L. C., J. Schaeffer, J.-C. L. Saux, S. Panaudeau, M. Elimelech and F. S. L. Guyader (2011). "Calicivirus removal in a membrane bioreactor wastewater treatment plant." Applied and Environmental Microbiology 77(15): 5170-5177.
4	Severn, R. (2003). "Long Term Operating Experience with Submerged Plate MBRs." Filtration and Separation 40(7): 28-31.
5	Pettigrew, L., M. Angles and N. Nelson (2010). "Pathogen removal by a membrane bioreactor." Journal of the Australian Water Association 37(6): 44-51.
6	(Hirani *et al.* 2012) Hirani, Z. M., J. F. DeCarolis, G. Lehman, S. S. Adham and J. G. Jacangelo (2012). "Occurence and removal of microbial indicators from municipal wastewaters by nine different MBR systems." Water Science & Technology 66(4): 865 - 871
7	Chiemcharisri, C., Y. K. Wong, T. Urase and K. Yamamoto (1992). "Organic Stabilisation and Nitrogen Removal in a Membrane Seperation Bioreactor for Domestic Wastewater Treatment." Water Science & Technology 25(10): 231 - 240
8	Xiang, Z., L. Wenzhou, Y. Min and L. Junxin (2005). "Evaluation of virus removal in MBR using coliphages T4." Chinese Science Bulletin 50(9): 862-867.
9	Lv, W., X. Zheng, M. Yang, Y. Zhang, Y. Liu and J. Liu (2006). "Virus removal performance and mechanism of a submerged membrane bioreactor." Process Biochemistry 41(2): 299-304.
10	DeCarolis, J. F. and S. Adham (2007). "Performance Investigation of Membrane Bioreactor Systems During Municipal Wastewater Reclamation." Water Environment Research 79(13): 2536-2550.
11	van den Akker, B., T. Trinh, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan (2014). "Validation of a full-scale membrane bioreactor and the impact of membrane cleaning on the removal of microbial indicators." Bioresoure Technology(0).
12	Marti, E., H. Monclús, J. Jofre, I. Rodriguez-Roda, J. Comas and J. L. Balcázar (2011). "Removal of microbial indicators from municipal wastewater by a membrane bioreactor (MBR)." Bioresource Technology 102(8): 5004-5009.
13	Trinh, T., B. v. d. Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan (2012). "Removal of endocrine disrupting chemicals and microbial indicators by a decentralised membrane bioreactor for water reuse." Journal of Water Reuse and Desalination 2(2): 67 - 73.
14	Validation of a full-scale Membrane Bioreactor for water recycling: Characterising process variability. Ozwater 2012. Sydney, Australia.

Pathogenic virus data surveyed included adenovirus (AdV), enterovirus (EV), norovirus (NoV) and sapovirus (SV) with 5th percentile LRVs, determined by method 3, of > 1.5, >1.3, >1.2 and >1.4 respectively. Readings above the detection limit in the permeate occurred for 66% (AdV), 31% (EV), 53% (NoV) and 6% (SV) of samples. Somatic coliphage and FRNA bacteriophage 5th percentile LRV, from the literature review, were 2.2 and 2.9. Somatic coliphage and FRNA bacteriophage were detected in the permeate in 60% and 85% of cases, from a data set 10 times larger than the pathogenic LRV data. More importantly, the lower LRVs for FRNA and SC were not censored with permeate non-detects, meaning, their extrapolation theoretically approaches a true value when compared to the limited case of the pathogenic viruses; the difference between method 2 and method 3 for data fitting.

The pathogenic virus data (Table 3) exhibited high standard deviations in a majority of distributions fitted by method 1. This was likely due to the seasonal variability of pathogens in wastewater resulting in significant concentration ranges when analysed over long periods (6 months – 1 year). Additionally, almost every source exhibited different detection limits as QPCR methods evolved from 2005 – 2012. When compared to indicator viruses, the pathogenic species data set is far smaller (one tenth of the values), presenting a further source of error.

Of the indicator viruses, MS2 bacteriophage (a subset of FRNA bacteriophages), and somatic coliphage demonstrated the highest resistance with 5th percentile LRVs of 1.8 and 2.2 respectively. Indigenous coliphage (reported as total coliphage) and somatic coliphage exhibited slightly higher mean LRV for smaller pore size membranes 0.04 µm (hollow fibre) compared to 0.4 µm (hollow fibre and flat sheet). However, the standard deviation of the smaller pore size membrane LRV data set was larger; to the point where 5th percentile LRVs were not significantly different (Table 4). Pathogenic and indicator virus data was combined as a whole data set (334 detected/597 total values) and yielded a 5th percentile LRV of 1.7.

For three of the lowest distributions, bacterial indicators were removed > 3.5 log, 95% of the time. Mean removal for all bacterial indicators ranged from 5 – 7 log units (Table 5). The combined set of all bacterial indicators (354 detected /684 total values) yielded a 5th percentile LRV of 4.6.

The protozoan indicator set was mainly comprised of *Clostridium perfringens* (Table 6) but included, three *cryptosporidium* and two *giardia* LRV, that were not detected in MBR permeate. More LRV data is required for protozoan removal by MBR. The combined set of all protozoan pathogens and indicators (21 detected / 26 total values) yielded a 5th percentile LRV of 3.5.

3.2 LRV from site visit data

3.2.1 Operating envelope

Operating data collected to correspond with LRVs for site sampling and from validation reports is summarised in Table 8. In addition, the subset of operating data to accompany only those sites with pore size < 0.1 µm was included in Table 9. Some values were not available, data set completeness, eg number of values of total possible, was included in the last column in order to express this. All sites investigated utilised membranes with reported nominal pore size of 0.04 µm, with the exception of site 1 and site 7 where pore sizes were 0.4 and 0.1. µm respectively (Table 1). The range of LRVs presented in the following section is considered valid under the operating conditions listed below.

Table 8 - Operating data from MBR site visits and validation reports all sites

Parameter	**Operating Data**							
	5th %ile	**25th %ile**	**Median**	**Average**	**75th %ile**	**95th %ile**	**N values**	**Data set complete (%)**
Bioreactor pH	5.2	6.6	6.9	6.8	7.0	7.5	250	95
Bioreactor DO (mg/L)	0.7	1.7	2.6	3.2	4.9	7.3	228	87
Bioreactor Temp. (°C)	16	20	22	22	5	29	223	85
SRT (d)	11	19	32	55	105	147	190	73
HRT (h)	6.5	11.8	19.7	23.0	32.4	44.9	201	77
MLSS (g/L)	1.9	4.4	5.4	7.0	9.7	14.5	229	87
MLVSS (g/L)	1.5	3.1	4.1	4.8	6.0	9.9	187	71
TMP (kPa)	2.3	3.7	6.4	10.3	7.8	47	308	85
Flux (L/m^2/h)	2.8	8.9	20.6	19.1	26.4	33.9	326	90
Permeability (L/m^2/h/kPa)	0.3	0.9	3.8	3.9	6.4	8.6	308	85
Turbidity (NTU)	0.01	0.03	0.05	0.13	0.13	0.37	263	73

Table 9 - Operating data from MBR site visits and validation reports where installed nominal pore size was specified as < 0.1 µm

Parameter	**Operating Data (only <** 0.1 µm)							
	5th %ile	**25th %ile**	**Median**	**Average**	**75th %ile**	**95th %ile**	**N values**	**Data set complete (%)**
Bioreactor pH	5.1	6.6	7.0	6.8	7.0	7.5	209	95
Bioreactor DO (mg/L)	0.9	1.7	2.5	2.8	3.3	5.7	187	85
Bioreactor Temp. (°C)	16	19	22	22	24	29	182	82
SRT (d)	11	19	36	55	99	147	149	67
HRT (h)	6	15	25	25	35	46	160	72
MLSS (g/L)	1.7	4.1	4.9	6.2	6.7	14.6	188	85
MLVSS (g/L)	1.3	2.8	4.0	4.7	6.0	9.9	164	74
TMP (kPa)	2.3	3.2	6.4	10.8	8.8	48.0	227	84
Flux (L/m^2/h)	2.5	5.9	19.8	15.6	22.2	28.8	245	91
Permeability (L/m^2/h/kPa)	0.2	0.8	1.6	3.4	6.6	8.4	227	84
Turbidity (NTU)	0.01	0.03	0.05	0.12	0.09	0.28	185	69

When considering the limited subset for membranes with installed pore size less than 0.1 µm, some key parameters were also adjusted within the operating envelope. For example when moving from the whole data set to the < 0.1 µm subset the 95th percentile turbidity and flux reduced from 0.37 to 0.28 NTU and 34 to 29 L/m^2/h, respectively.

3.2.2 Indicator Removal

Indicator LRV collected from site visits and validation reports were summarised into PDFs using data fitting method 1. LRV PDFs are shown for CP, EC, SC and FRNA in Figures 1, 2, 3 and 4, respectively.

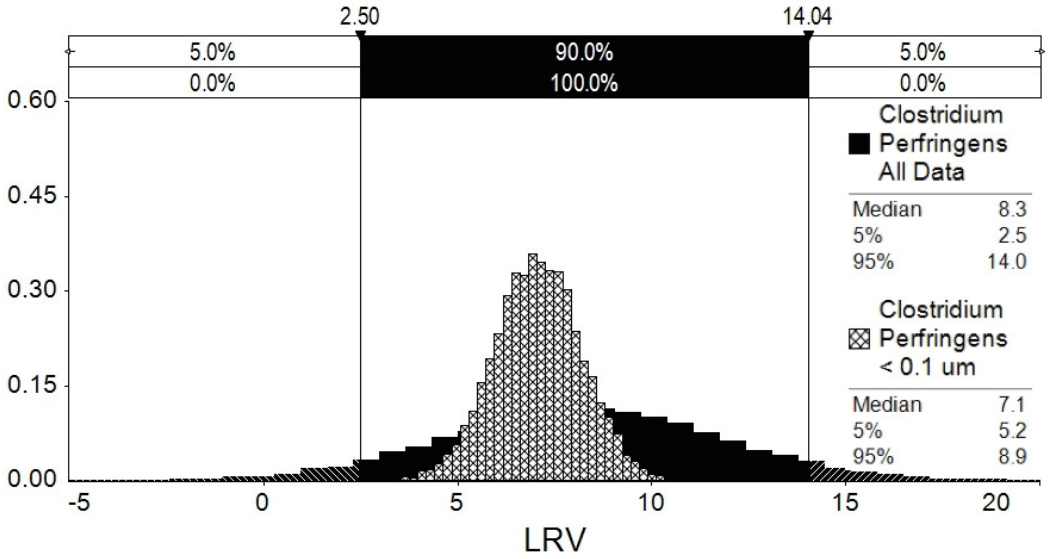

Figure 1 – Clostridium perfringens LRV PDFs from site visits and validation reports comparing the entire data set with the < 0.1 µm pore size subset

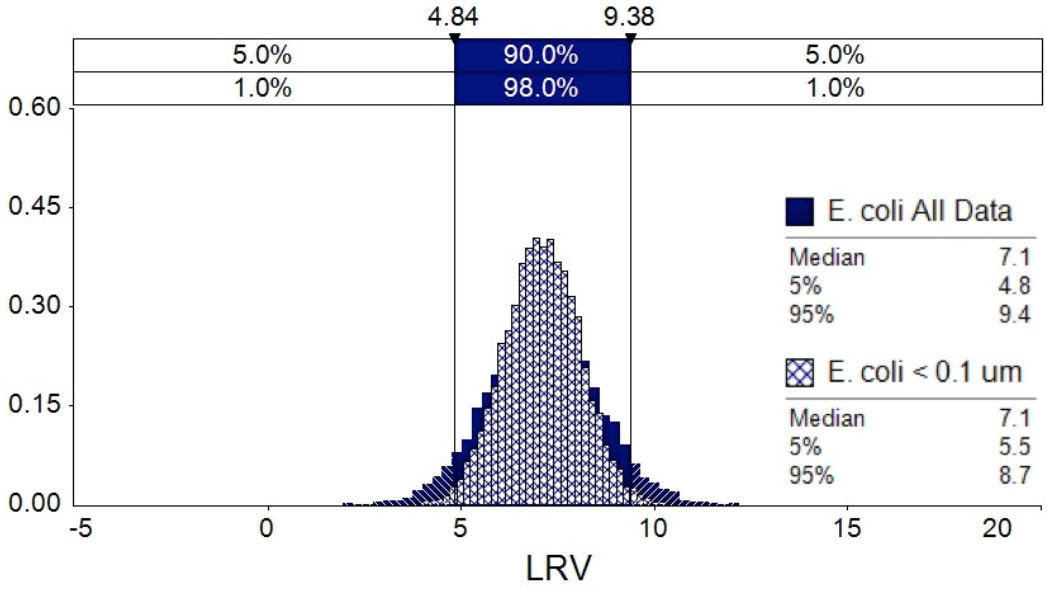

Figure 2 – E. coli LRV PDFs from site visits and validation reports comparing the entire data set with the < 0.1 µm pore size subset

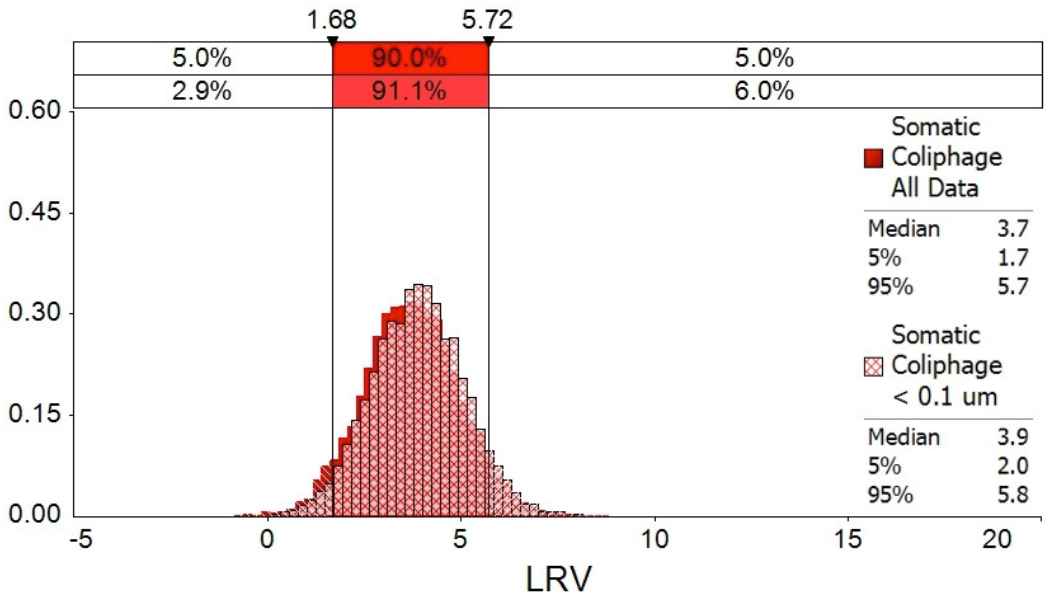

Figure 3 – Somatic coliphage LRV PDFs from site visits and validation reports comparing the entire data set with the < 0.1 µm pore size subset

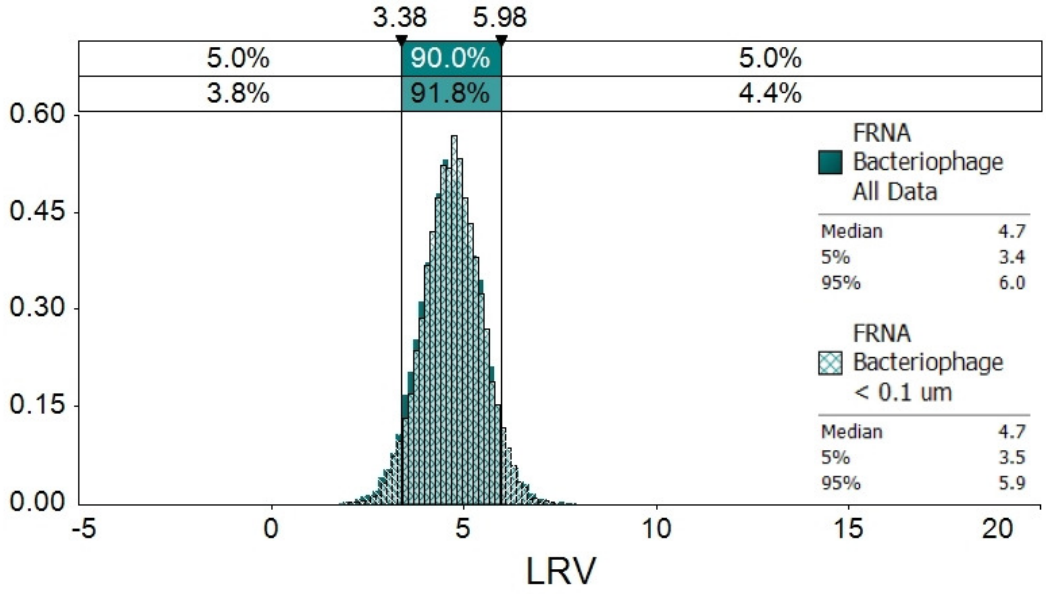

Figure 4 - FRNA bacteriophage LRV PDFs from site visits and validation reports comparing the entire data set with the < 0.1 µm pore size subset

For the entire data set of CP, the LRV PDF featured a high standard deviation. This was likely a result CP accumulation within the bioreactor (increase by 1.4 log), meaning that if breakthrough occured a higher permeate density would be observed. The subset for membranes installed with a nominal pore size of less

than 0.1 µm exhibited a much lower standard deviation with a 5th percentile of 5.2, almost double that for the entire data set (5th %ile LRV for All CP was 2.5) (Figure 1).

EC was removed substantially by the MBR with a 5th %ile of 4.8 for the total data set and 5.5 for membranes with pore sizes of < 0.1 µm. The higher removal of EC when compared to CP was likely due to the contribution of the bioreactor to LRV, which reduced EC densities by 0.9 log from influent to activated sludge (Figure 2). Interestingly the median value (7.1) was identical for both data sets, which may imply that the smaller pore size reduced removal variability without shifting performance to appreciably higher values.

SC LRV 5th %iles were 1.7 and 2.0 for the total data set and < 0.1 µm subset, respectively. Similarly, FRNA exhibited little change when observing the subset of smaller pore sizes with 5th percentile LRVs of 3.4 and 3.5. Even though wild SC can exhibit much larger variation in size than FRNA, it would appear this is not a primary factor governing observed removal. Rather, the resistance of SC to biological treatment resulted in a greater density against the membrane leading to a higher rate of detection in the permeate, with 72% of permeate samples above the LOD. By contrast, FRNA bacteriophage median removal through the bioreactor was 1.2 log units and was only detected in 20% of permeate samples (Figure 3 – 4). The small impact of limiting pore size is partially explained as even if 0.04 µm membranes are used FRNA (c. 0.025 µm) and SC (0.027 – 0.100 µm) could still be expected to pass if removal was due to size exclusion alone.

3.2.3 Pathogen removal

Samples were taken from sites 3 and 9 and analysed for *cryptosporidium, giardia*, enteroviruses, adenoviruses and reoviruses alongside normal sampling.

Cryptosporidium and *giardia* were not detected in any of the six 100L permeate samples analysed across sites 3 and 9. Overall LRV for cryptosporidium and giardia varied between > 3.1 - > 4.1 and > 4.3 - > 6.1, respectively. Removal

within the activated sludge for *cryptosporidium* was -1.2 – 0.4 log units and for *giardia* was between -0.9 – 0.7 (Figures 5 and 6). CP was proposed as a surrogate for *cryptosporidium.* As neither CP or protozoan pathogens were detected in the permeate, direct correlation of CP overall LRV with cryptosporidium and giardia was not possible. Overall CP LRV varied between > 5.2 - > 5.6. LRV_{Bio} of cryptosporidium and giardia did not correlate significantly with CP. Removal in the bioreactor for CP was between – 1.7 and – 0.8, accumulating and more conservatively removed when compared to *cryptosporidium* and *giardia*. Even though CP removal did not correlate, it was shown to be more conservatively removed within the bioreactor than either protozoa. Given CP comparatively greater resistance to biological treatment and that it is 5 – 10 times smaller than either protozoa the use of CP as a conservative indicator is still valid.

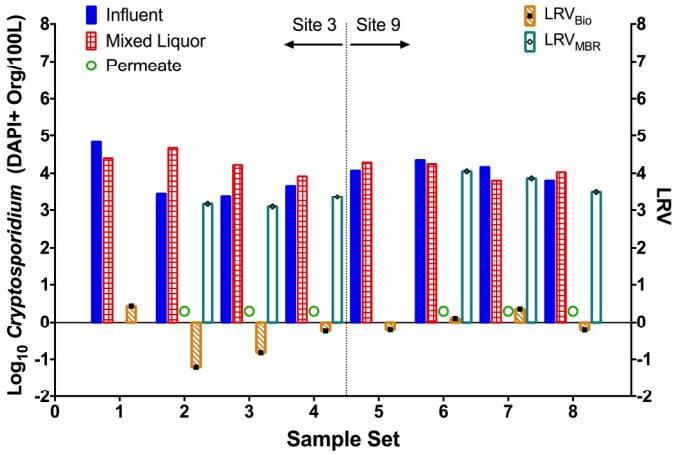

Figure 5 – Cryptosporidium densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

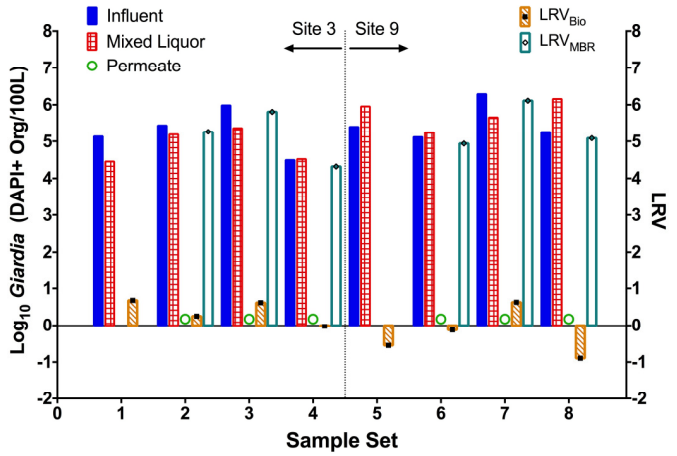

Figure 6 - Giardia densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

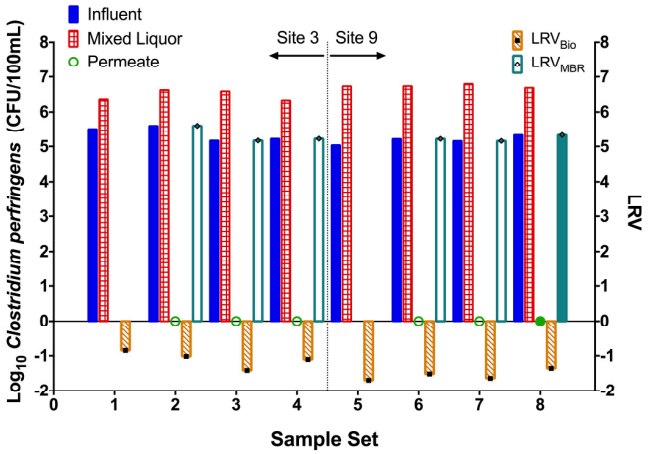

Figure 7 – Clostridium perfringens densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

Enterovirus, adenovirus and reovirus LRV varied between >3.3 - >5.1, >4.0 - >5.3 and >3.3 - >4.7 (Figures 8 – 10). No viruses were detected in the six 100L permeate samples taken for analysis. For the same set of samples SC and FRNA LRV were between 2.9 – 4.2 and >3.2 - >4.2, respectively (Figures 11 – 12). Correlations between both virus indicators and pathogens were poor, however FRNA followed the same trend of positive removal within the bioreactor. Even though correlations were poor, SC were more conservatively removed and more readily detected in the permeate than the corresponding virus indicators.

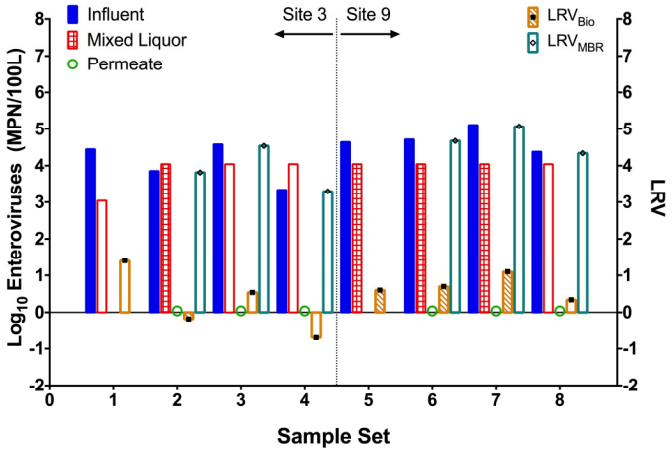

Figure 8 - Enterovirus densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

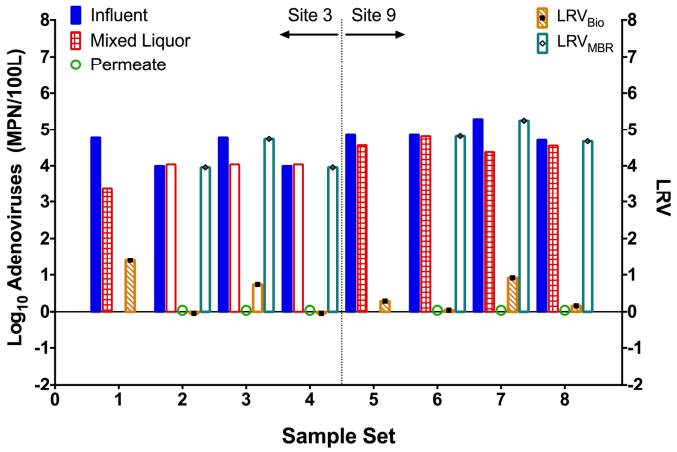

Figure 9 - Adenovirus densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

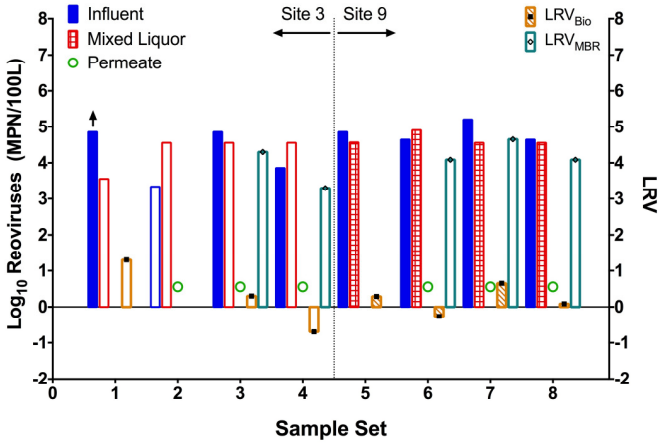

Figure 10 - Reovirus densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively. An arrow indicates a density above the reporting limit.

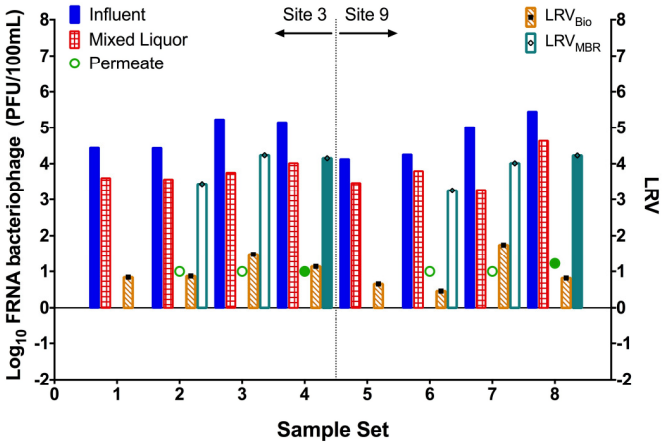

Figure 11 – FRNA bacteriophage densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

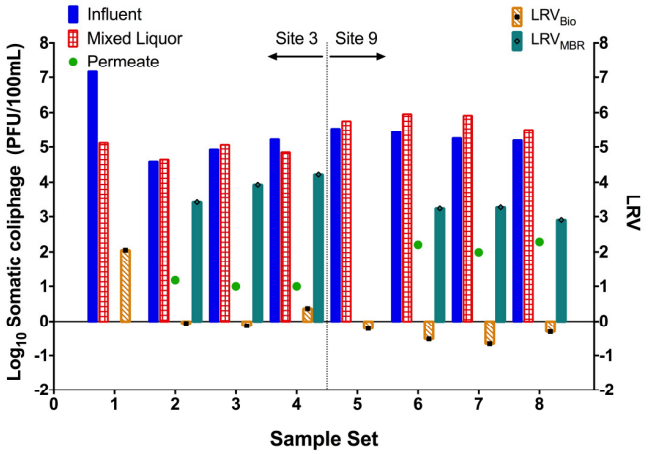

Figure 12 – Somatic coliphage densities and LRV. Open symbols and bars denote permeate below the LOD and greater than LRVs, respectively.

Poor correlation of indicators with pathogens was noted. This may be due to the much higher variability of wastewater densities of pathogens when compared to indicators. Further sampling could be conducted in order to permit a more robust assessment with Monte Carlo techniques. For the virus indicators more conservative overall removal was noted. As such, it is proposed that either virus indicator could be chosen in place of the pathogens with the expectation of a more conservative LRV. There was evidence to suggest more conservative removal in the activated sludge and also a significantly smaller size of CP compared to protozoa. Accordingly, CP is still suggested as a viable surrogate for a more conservative result.

Even though correlation of pathogens and indicators was poor, overall removal was appreciable. Worst case protozoan and virus removal was noted for *cryptosporidium* and enteroviruses with LRVs of >3.1 and >3.3, respectively.

4 Decision on a default value

In order to establish a default value, a large body of literature and full scale site data was considered. From the data collected, either from full scale sites or literature, a worst case LRV was determined.

The 5th percentile overall virus LRV of 1.7 from literature corresponded with that of somatic coliphages from full-scale site data, lower than the > 3.3 observed as the minimum enterovirus LRV. Pathogenic virus data surveyed included adenovirus (AdV), enterovirus (EV), norovirus (NoV) and sapovirus (SV) with 5th percentile LRVs, determined by method 3, of > 1.5, >1.3, >1.2 and >1.4 respectively. As method 3 was used these values are highly conservative and were believed to be incorporated adequately by rounding down indicative virus LRVs. The limited impact of pore size on virus removal was noted with an increase in only 1.7 to 2.0 by excluding pore sizes > 0.1 µm. 5th percentile protozoan LRV indicated by *clostridium perfringens* was 2.5, lower than the > 3.1 observed for cryptosporidium LRV minimum. When pore size was limited the 5th percentile for *clostridium perfringens* increased significantly to 5.2. If smaller pore size membranes are employed an increased log removal credit (to a maximum of 4) may be valid for larger protozoa. *E. coli* LRV from full scale site data was 4.8 at the 5th percentile for the entire data set and 5.5 for the < 0.1 µm pore size subset . For all bacteria LRV isolated from literature, the 5th percentile was 4.6. Given that a maximum LRV of 4.0 per unit operation is adopted to encourage multiple barriers in a reuse scheme, bacteria removal does not appear to be a concern for MBR.

A further conservative step was introduced whereby the LRV was rounded down to the nearest half log.

5 Conclusions

Provided a MBR is operated within the range of parameters assessed in Table 8, in particular turbidity should not exceed 0.4 NTU at the 95th percentile, the following default LRV, rounded down to the nearest half log are proposed for MBR:

Virus: 1.5

Bacteria: 4.0

Protozoa: 2.0

If further limitations are imposed, such as installing membranes with a pore size < 0.1 µm, not exceeding a flux of 30 L/m^2/h or permeate turbidity at the 95th percentile of 0.3 NTU then the following default values may be valid:

Virus: 1.5

Bacteria: 4.0

Protozoa: 4.0

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Appendix A – MBR LRV from Literature

See attachment

Appendix B – MBR Data Sheet

See attachment

Appendix C – Data fitting methods

Fitting Method 1

Data Requirements

- Raw concentrations (Feed, Permeate and Activated Sludge)
- Knowledge of the detection limit for each set
- At least 3 values in each set to be used above the detection limit.

Method

Each independent set is sorted from lowest to highest value.

Each point in the set is assigned a 'p-value' with the blom formula (Equation 1)

$$p = \frac{(i-0.375)}{(n+0.25)} \qquad \text{Equation 1}$$

Where: p is the resulting p value (between 0 – 1), i is the order of the point in the data set (eg the first point is i = 1, second point is i = 2) and, n is the total number of points in the set.

All points above the limit of detection and their p values are highlighted and a log normal distribution is fitted using @risk software (Palisade 2013) (Figure 13).

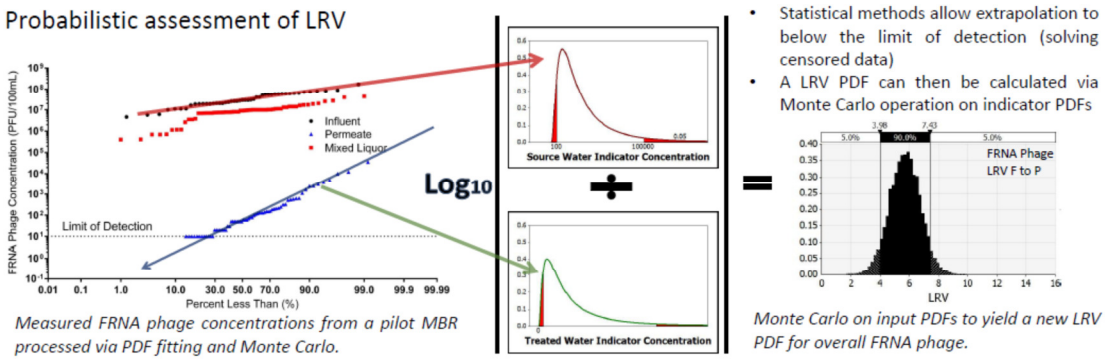

Figure 13 - Illustration of Method 1. Note 30% of permeate FRNA phage were below the detection limit, hence only the higher 70% have been used in @risk to create the intermediate distribution.

Table 10 - Advantages and disadvantages of fitting Method 1.

Advantages	Disadvantages
Efficiently deals with censored LRV values e.g. > LRV	Appears to yield broader (higher standard deviation) distribution than a paired LRV analysis of the same data set.
	Data often not provided as raw concentrations
	Cannot be used to combine across different site data due to step changes in feed distribution

Fitting Method 2

Data Requirements

- LRV data set
- Knowledge of whether or not the paired LRV is the true value (detected in the permeate) or censored e.g > LRV
- At least 3 of the lowest LRV must be uncensored

Method

LRV are sorted from lowest to highest and assigned a p value similarly to the concentration data points in method 1.

All of the lower LRV values and their p values, before the first non-detect are fitted to a normal distribution in @risk (Figure 14).

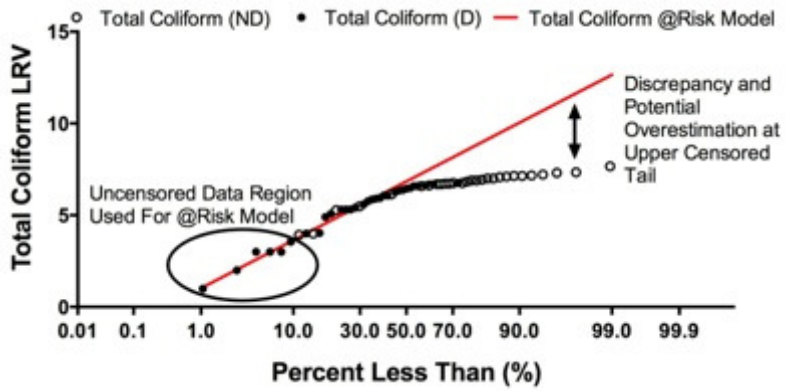

Figure 14- Method 2 fitting procedure. Note feed water changes combined with removal performance variation means that there may be censored LRVs dispersed among the set of absolute LRV.

Table 11 - Advantages and disadvantages of fitting Method 2.

Advantages	Disadvantages
Provides an LRV distribution calibrated to conservative lower values.	No way of assessing if performance is over estimated in upper censored region.
Can be used to combine sets of LRV from different sources/sites (performance not dependant on feed distribution).	For larger organisms (bacteria and protozoa) with MBR often there are not > 3 uncensored data points at the lower end of the distribution.

Fitting Method 3

Data Requirements

- LRV data set

Method

LRV are sorted from lowest to highest and assigned a p value similarly to the concentration data points in method 1.

All of the LRV values and their p values are fitted to a normal distribution in @risk (Figure 15). For comparison the same data set for Figure 14 has been utilised.

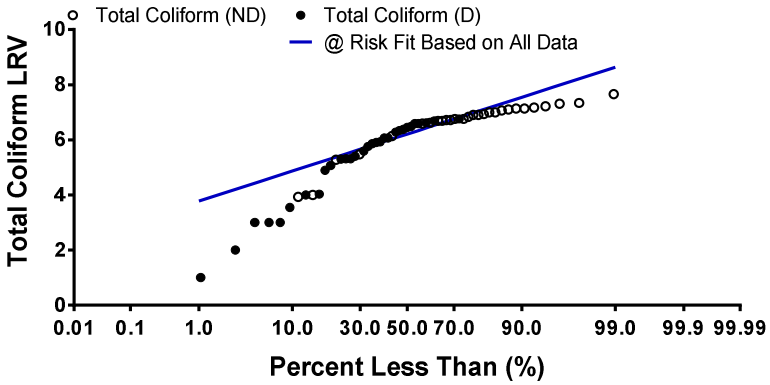

Figure 15 – Method 3 Fitting Procedure. Note the LRV although now based on more data points exhibits a higher 5th percentile, similar average and lower higher percentile performance.

Theoretically method 3 should result in a conservative distribution as it assumes the upper bounded LRVs are a true value.

Table 12 - Advantages and disadvantages of fitting method 3

Advantages	Disadvantages
Provides an LRV distribution calibrated to a large number of reported values.	Possibility of over estimating lower percentile performance.
Can be used to combine sets of LRV from different sources/sites (performance not dependant on feed distribution).	Ignores the effect of censored data.
	Non normality (of assuming a censored value is true) can result in curvature and poor model fit.