

GLOBAL WATER PATHOGEN PROJECT

**PART FOUR. MANAGEMENT OF RISK FROM EXCRETA AND WASTEWATER**

# **MEMBRANE BIOREACTORS**

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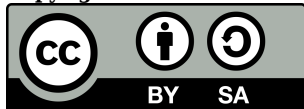
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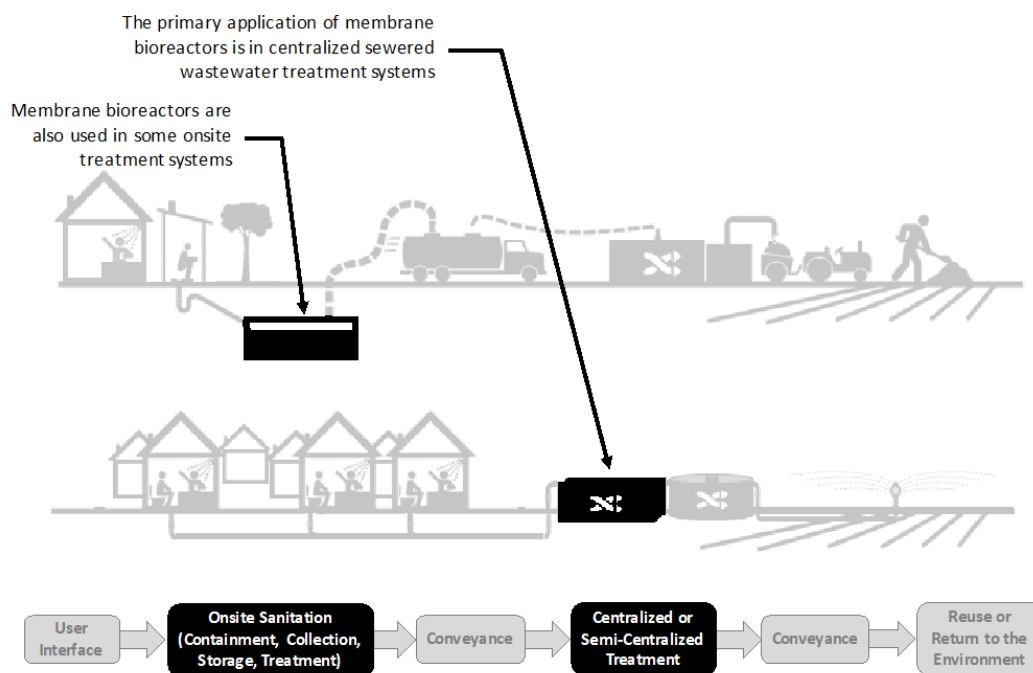
## Summary

Membrane bioreactors (MBRs) are a sanitation technology used in centralized wastewater treatment facilities and, infrequently, in some onsite systems. They are similar to activated sludge systems, but utilize micro- or ultra-filtration membranes instead of secondary clarifiers to remove bacterial biomass from secondary wastewater. Some pathogen reduction is achieved in MBRs during biological treatment, but a much greater reduction is achieved as the mixed liquor passes through the membrane. The pore sizes of membranes typically used in these systems will retain most bacteria, but are large enough for viruses to pass through. However, the smaller effective pore sizes due to the development of a cake layer considerably increase the efficiency of virus removal in these systems. Under normal operating conditions, on average, these systems achieve 4.4- $\log_{10}$  reduction of bacterial pathogens and 3.3- $\log_{10}$  reduction of viral pathogens. Some of the most important factors influencing pathogen reduction efficiency in MBRs include the ratio of pathogen size to membrane pore size, the extent of membrane fouling, variations in feed water, membrane integrity, solids retention time and hydraulic retention time. The efficiency of pathogen reduction in MBR systems will also undoubtedly be influenced by local conditions, including socioeconomic and geographical factors that affect a community's ability to provide proper maintenance

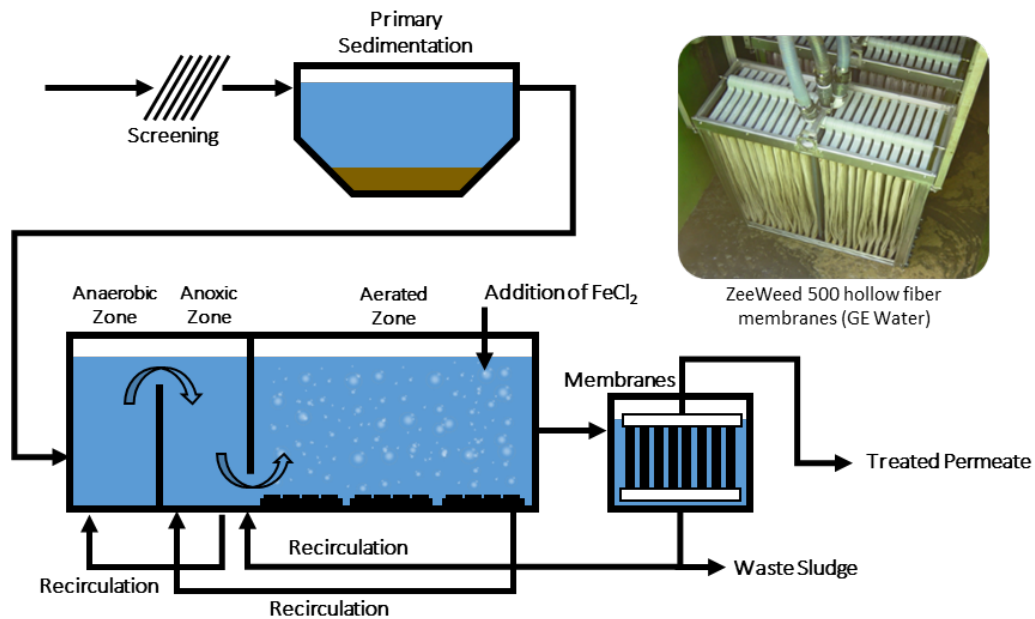
to these systems. MBR is a complex treatment process that should be intensively validated before assigning  $\log_{10}$  reduction credits. Any  $\log_{10}$  reduction values reported herein should require local validation prior to being used for regulatory purposes. Additionally, the sludge wasted from MBR systems likely contains high concentrations of pathogens, although this has not been well studied. MBR sludge should be treated to further reduce pathogens prior to reuse or disposal.

### 1.0 Brief Technology Description

Membrane bioreactors (MBR) are a sanitation technology generally used in centralized wastewater treatment facilities, and variations of this technology are also used in onsite systems as retrofits or modifications to conventional septic systems (Chiemchaisri et al., 1993) (Figure 1). An MBR system is a modification of activated sludge, with micro- or ultra-filtration membranes used instead of secondary clarifiers to remove bacterial biomass from the treated effluent. MBR systems may have membranes that are external (located outside the aeration tank) or immersed (located inside the aeration tank). These systems commonly operate with solids retention times of 20 to 30 days and hydraulic retention times of 6 to 10 hours. Membranes can be made from mineral or organic materials, and typically have tubular, hollow fiber, or plate-like shapes. Wastewater is passed through the membrane under vacuum conditions or using hydrostatic pressure. Figure 2 shows a schematic of a wastewater treatment system in France that utilizes the MBR technology.



**Figure 1. Locations where the membrane bioreactor technology is used within the sanitation service chain.** This image is a derivative of "Sanitation Value Chain" by SuSanA Secretariat, which is licensed under CC BY 2.0.



**Figure 2. Schematic of an MBR system with an external membrane, based on the Seine Morée Wastewater Treatment Plant in France, which is operated by the Conurbation of Paris Sanitation Authority (SIAAP), and serves a population of 300,000 residents.** The plant uses the ULTRAFOR® membrane bioreactor process with ZeeWeed 500 hollow fiber membranes (see photo inset), which have a cut-off threshold of 0.04  $\mu\text{m}$ . Approximately 62% of flow from the membrane gets recirculated back to the aerobic chamber, while 18% of flow from the aerobic chamber recirculates to the anoxic chamber, and 16% of flow from the anoxic chamber circulates back to the anaerobic chamber. (photo credits SIAAP-DDP).

## 2.0 Inputs and Outputs of Membrane Bioreactors

MBRs are typically used to treat wastewater from domestic sources, or from a mixture of domestic and industrial sources. Figure 3 shows the typical input and output of a MBR system. The influent to the membrane of an MBR system may have already passed through a primary clarifier, often followed by sieving or fine screening, and then some secondary treatment in the activated sludge aeration tank. Therefore, the concentrations of pathogens entering the membrane may be slightly lower than they were in the untreated wastewater. However, pathogens in the return activated sludge can actually cause the concentration at the

influent of the membrane to be greater than the concentration in the untreated wastewater (Simmons et al., 2011). Typical concentrations for pathogens in the liquid and/or solid inputs to MBR systems are provided in the Introduction.

The outputs from MBR systems include treated water effluent and sludge. The treated effluent is continuously discharged. The sludge, generated from the process of membrane fouling, accumulates over time and is typically removed every few weeks. This sludge is often mixed with sludge from the primary clarifier, and must be treated prior to reuse or land application (see Chapter on Sludge Management). MBR systems with anaerobic units can also produce biogas that can be harvested and reused for energy production.

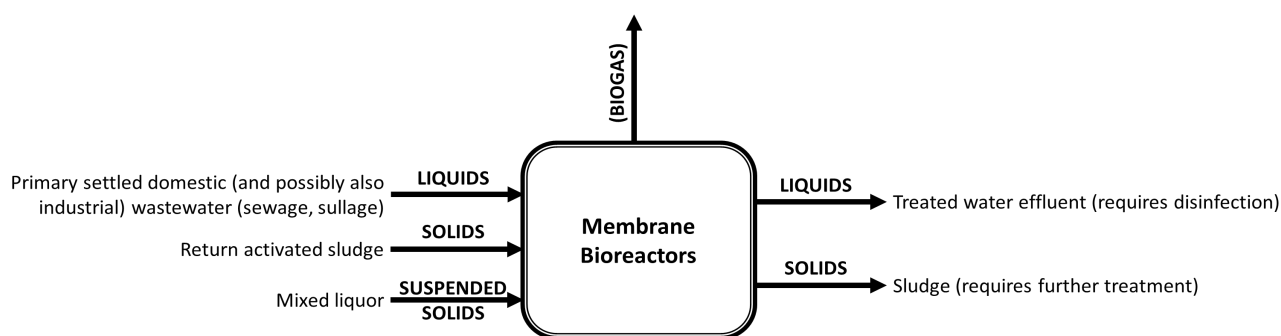


Figure 3. Typical inputs and outputs from membrane bioreactor systems.

### 3.0 Factors Affecting Pathogens in Membrane Bioreactor Systems

MBR systems usually include primary treatment, and some pathogen reduction is achieved in primary clarifiers where larger pathogens and pathogens associated with larger particles settle out of the wastewater and are transferred to the primary sludge (see chapters on Preliminary Treatment and Primary Sedimentation). After primary treatment, pathogen concentrations in MBR systems are reduced by a combination of inactivation (loss

of viability) and removal (retention in the sludge) (Tables 1a and 1b). Predation is the main mechanisms of pathogen reduction during biological treatment, and size exclusion is the main mechanism of pathogen reduction by the membrane. The efficiency of pathogen reduction via predation during biological treatment is 4 to 7  $\log_{10}$  units lower than the reduction achieved via size exclusion by the membrane (Branch et al., 2016a). However, activated sludge flocs are also formed during biological treatment, and the presence of pathogens attached to or enmeshed within these flocs also affects how well they are removed by the membrane (Hai et al., 2014).

**Table 1a. Factors and mechanisms for pathogen reduction in membranebioreactors: Loss of viability**

Factors affecting this mechanism	Evidence of Pathogen Vulnerability
<p>Pathogens lose viability in the mixed liquor due to predation and other microbiological factors (Branch et al. 2016a; Chaudhry et al. 2015a). Well-developed cake layers also host a large consortium of macro- and microorganisms, and some biological inactivation may occur there as well. Pathogen reduction efficiency in the mixed liquor is affected slightly by operational factors such as the solids retention time and the hydraulic retention time (Sidhu et al. 2015), and also by variations in feed water characteristics (Branch et al. 2016b)</p>	<p>In a full-scale MBR systems using the modified Ludzack-Ettinger process, Chaudhry et al. (2015a) estimated that the inactivation of adenovirus, norovirus GII, and F+ bacteriophage in the mixed liquor due to predation and enzymatic decay contributed only <b>1.1 to 1.7 <math>\log_{10}</math></b> units of virus reduction, compared to the overall reductions of <b>&gt;5 <math>\log_{10}</math></b> units</p> <p>Branch et al. (2016a) reported accumulation of <i>C. perfringens</i> spores (a protozoan surrogate) during the biological process of an MBR system</p> <p>Reduction of <i>E. coli</i> in the mixed liquor of an MBR system (presumably due to predation and enzymatic decay) was only <b>0.5 to 1.1 <math>\log_{10}</math></b> units (Branch et al. 2016a)</p>

**Table 1b. Factors and mechanisms for pathogen reduction in membrane bioreactors: Size exclusion and retention by the membrane and cake layer.**

Factors affecting this mechanism	Evidence of Pathogen Vulnerability		
	Viruses	Bacteria	Protozoa and Helminths
Retention (size exclusion) is the primary mechanism by which pathogens are removed from wastewater in MBR systems (Chaudhry et al. 2015a). The efficiency with which pathogens are retained at the membrane is affected by the following factors: The ratio of the nominal pore size of the membrane relative to the dimensions of the pathogen Development of the cake layer on the membrane (time elapsed after start-up or after the routine cleaning of the membrane) The following factors influence pathogen adsorption to suspended solids in the mixed liquor (Stevik et al. 2004), which facilitates retention by the membrane or the cake layer: Concentration/composition of organic matter Salt content (ionic strength) pH Temperature Bacterial activity in mixed liquor	With diameters of ~25 nm (norovirus) to ~100 nm (adenovirus), many enteric viruses are smaller than the nominal pore size of membranes used in most MBR systems. Chaudhry et al. (2015a) reported that retention by a 0.04µm membrane or its cake layer accounted for >50% of the total log <sub>10</sub> reduction values for adenovirus, norovirus GII, and F+ phage, and that >1.0 log <sub>10</sub> of these viruses were attached to mixed liquor suspended solids, facilitating retention	Most bacterial pathogens have diameters of 0.3 µm or larger and are thus larger than the nominal pore size of membranes used in most MBR systems. Branch et al. (2016a) reported 6.4 to 6.7 log <sub>10</sub> removal of <i>E. coli</i> from the membrane alone; removal decreased to 4.7 log <sub>10</sub> units for a membrane that was at the end of its useable life (10 years)	The (oo)cysts and eggs of protozoan and helminth parasites generally have diameters of 4µm or more, making them much larger than the nominal pore size of membranes used in most MBR systems. Helminth eggs have never been detected in the effluent of neither of two MBR systems located along the Seige River in France (authors' own unpublished data). Branch et al. (2016a) reported >6 log <sub>10</sub> removal of the spores of <i>C. perfringens</i> (a protozoan surrogate) from the membrane alone

**3.1 Inactivation by Predation or Enzymatic Activity**

Just like in conventional activated sludge systems, pathogens are reduced during biological treatment process in MBR systems. This reduction is primarily attributed to natural decay, predation by organisms of higher trophic levels, and the production of proteolytic enzymes by the consortia of microorganisms in the mixed liquor (Kim and Unno 1996; Hao et al. 2010; Sidhu et al. 2015). See the Activated Sludge chapter for more details about the factors affecting pathogens during biological treatment.

Greater adsorption of pathogens to suspended solids leads to more efficient removal at the membrane (see Section 3.2). There are several factors related to the influent water quality that can affect adsorption of pathogens to mixed liquor suspended solids, such as the concentration and composition of organic matter, salt content, pH, ambient temperature, and bacterial density (Stevik et al., 2004). Design and operational factors such as longer hydraulic retention times (George et al., 2002) and the use of alternating anoxic/oxic reactors, as in the Bardenpho process (Schmitz et al., 2016), can also improve pathogen

removal efficiency.

Chaudhry et al. (2015a) estimated that virus decay due to predation and enzymatic activity alone was responsible for 1.1 to 1.7 log<sub>10</sub> of the overall 5.5 to 7.1 log<sub>10</sub> removal in a full-scale MBR system with a pore size of 0.04 µm. Likewise, Branch et al. (2016a) reported negligible to 0.9 log<sub>10</sub> unit reduction of FRNA and somatic coliphages during biological treatment, with an additional 3.2 to 4.4 log<sub>10</sub> unit reduction provided as the mixed liquor passed through the membrane. The same authors reported <1.1 log<sub>10</sub> unit reductions of *E. coli* (rod-shaped bacteria, 1-2 mm long, 0.25-0.5 mm wide) during biological treatment, but an additional 4.7 to 6.7 log<sub>10</sub> unit reduction of *E. coli* as the mixed liquor passed through the membrane. The concentration of *Clostridium perfringens* spores actually showed an apparent increase in concentration during biological treatment (Branch et al., 2016a), but were consistently reduced at the membrane by more than 6 log<sub>10</sub> units.

### 3.2 Removal by Size Exclusion at the Membrane and Cake Layer

The primary mechanism for pathogen removal as the mixed liquor passes through the membrane is size exclusion, which is enhanced by the development of the biological cake layer that develops on the mixed liquor side of the membrane (Chaudhry et al., 2015a; Chaudhry et al., 2015b). Some pathogens retained by the membrane or cake layer may also lose viability due to predation or the production of proteases or nucleases by other microorganisms in the biofilm (Bosch et al., 2006; Chaudhry

et al., 2015a). It should be noted, however, that in other settings, biofilms have been shown to offer protection to some bacterial pathogens (e.g. *V. cholerae*) from predation by larger microorganisms (Matz et al., 2005). It should be assumed that most of the pathogens retained at the membrane will be transferred to the secondary sludge where they may remain viable and must be further treated (see Sludge Management chapter). In general, the viability of pathogens recovered in full-scale MBR system secondary sludge has not been well studied.

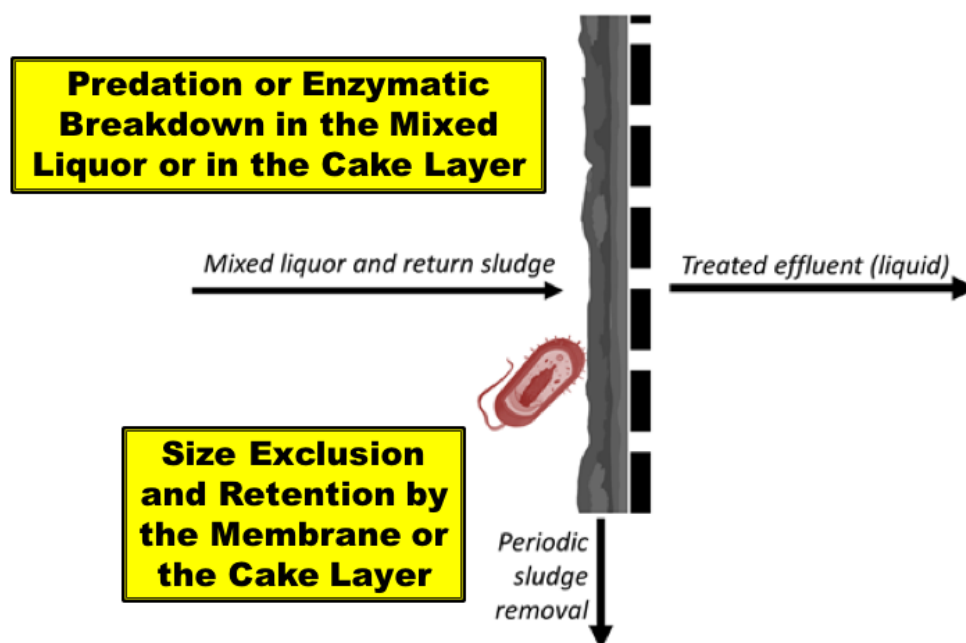


Figure 4. Major factors affecting pathogen removal in membrane bioreactors.

The pore sizes of most membranes used in practice are not small enough to physically remove viral pathogens by size exclusion alone; most MBR systems in practice employ microfiltration (0.1- to 0.4- $\mu\text{m}$  nominal pore size) or ultrafiltration (nominal pore size of 0.01- to 0.04- $\mu\text{m}$ ) (Hai et al., 2014). However, membranes in MBR systems with larger pore sizes have still been shown to reduce the concentration of viruses by 3 to 4  $\log_{10}$  units, due to the smaller effective pore sizes and adsorption in cake layer (Lv et al., 2006; Chaudhry et al., 2015a). Chaudhry et al. (2015a) reported an overall removal of 5.5  $\log_{10}$ , 5.6  $\log_{10}$ , and 7.1  $\log_{10}$  for adenovirus (measured via qPCR), norovirus GII (measured via RT-qPCR), and F+ coliphage (measured via plaque assay), respectively, in a full-scale MBR system after a 4- to 5-day cake layer was developed. The authors determined that the development of the cake layer was responsible for a 1.6  $\log_{10}$  increase in the removal of adenovirus and F+ coliphage, and a 0.4  $\log_{10}$  increase in the removal of norovirus GII. As such, virus removal efficiency in MBR systems may decrease immediately after the membranes are cleansed or treated for routine operation and maintenance (Lv et al., 2006).

Membranes have a distribution of pore sizes—the nominal pore size typically refers to a filter that is capable of cutting off a certain percentage by weight (e.g. 90%) of

glass beads with diameters greater than the specified size. Therefore, a small percentage of pores on the membrane will be larger than the nominal pore size, which explains the fact that pathogens larger than the nominal membrane pore size are sometimes detected on the downstream side of the membrane. It should also be noted that some bacteria may undergo deformation mechanisms, allowing them to pass through membranes with pore sizes smaller than the cell size (Gaveau et al., 2017). Differences in the thickness and elasticity of the cell wall enable Gram negative bacteria to have greater deformability than Gram positive bacteria. Figure 5 shows the  $\log_{10}$  reduction of viruses and fecal indicator bacteria in MBRs with respect to the ratio of the size of the microorganism to the nominal pore size of the membrane. For ratios greater than 1, the  $\log_{10}$  removal increases as the ratio increases. However, for ratios below 1 (i.e. viruses that are smaller than the nominal pore size of the membrane), the removal is not correlated with this ratio, and likely depends on other factors such as the development of the gel and cake layers and the transmembrane pressure.

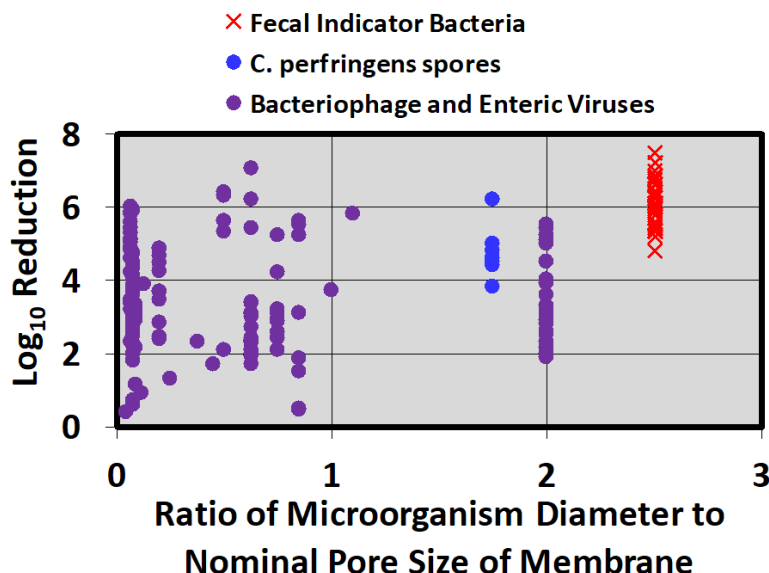


Figure 5. Virus and fecal indicator bacteria removal efficiency in membrane bioreactors with respect to the ratio of microorganism size to nominal pore size of the membrane (data sources: Branch et al., 2016b; Chaudhry et al., 2015a; Chaudhry et al., 2015b; Francy et al., 2012; Hai et al., 2014; Kuo et al., 2010; Lv et al., 2006; Marti et al., 2011; Purnell et al., 2016; Shang et al., 2005; Simmons et al., 2011; Wu et al., 2010; Zanetti et al., 2010; Zhang and Farahbakhsh 2007; Zheng and Liu, 2006).

#### 4.0 Design, Operation and Maintenance Guidelines for Pathogen Removal

Table 2 includes a summary of the key factors that

affect pathogen removal in MBR systems. Some of the most critical design and operational factors, maintenance issues, and malfunction concerns that will affect pathogen removal efficiency in MBR systems:

Table 2. Summary of key factors affecting pathogen removal in membrane bioreactor systems

Factor	Pathogen removal is ↑ enhanced or ↓ reduced under the following conditions:
Membrane Pore Size and Pathogen Size	Larger Pathogen to Pore Size Ratio = ↑ Pathogen Removal
Membrane Age and Integrity	Higher Pressure Decay Rates = ↓ Pathogen Removal Older Membrane = More Fouling* = ↑ Pathogen Removal (especially viruses)
pH	Lower pH = ↑ Pathogen Removal (especially viruses)
Feed Water Variation	Short-Term Ammonia or Salinity Shock = ↓ Pathogen Removal (temporary)

**Note:** an increase in transmembrane pressure or a decrease in flux are indicators of membrane fouling, which may increase virus removal efficiency, but can cause other types of operational problems; Sources: Branch et al., 2016b; Hai et al., 2014

**Membrane Pore Size and Pathogen Size.** The nominal pore size of the membrane impacts the efficiency of pathogen removal for MBRs. Pathogen removal becomes more efficient and consistent when the ratio of the diameter of the pathogen to the nominal pore size of the membrane is 2.0 or higher (Figure 5).

**Membrane Fouling.** Membrane fouling is the most challenging issue in the operation of MBRs (Meng et al., 2017) and it generally (but not always) increases pathogen reduction efficiency in ways that are not yet fully understood. This is because fouling itself is a very complicated issue that is still not yet fully understood and is an active topic of current research (Wang et al., 2014; Meng et al., 2017). An increase in the transmembrane pressure or a decrease in the flux across the membrane is



an indication of membrane fouling. Increased virus removal efficiencies have been reported at higher transmembrane pressures in bench-scale systems operating under constant flux (Zanetti et al., 2010; Marti et al., 2011; Yin et al., 2016). However, Yin et al. (2015) reported that the level of fouling alone is not a good predictor for virus removal, and that pore blockage by dissolved species increases virus removal but the cake layer may either increase or decrease virus removal efficiency depending on its properties.

- Reversible fouling is most commonly caused by polysaccharides, and can often be removed by mechanical cleaning (e.g., applying a shear force by scouring with air diffusers or backwashing with permeate) or by mild chemical cleaning (e.g., washing or soaking membranes with a mixture of permeate and reagents such as bleach and NaOH to eliminate the organic fraction of the clogged material, or citric acid and hydrochloric acid to eliminate the mineral fraction of the clogged material)
- Irreversible fouling is caused by a combination of chemical adsorption (e.g., non-covalent interactions between humic substances, polysaccharides and proteins) and pore clogging (Guo et al., 2012; Meng et al., 2017), and it may be partially removed in the short term by chemical cleaning; however, long-term fouling of this type may never be recovered, leaving a remaining resistance that may ultimately require membrane replacement (Wang et al., 2014)
- Some novel fouling control strategies include the use of bacterial enzymes or bacteriophages, electrically-assisted mechanisms, and the use of nanomaterial-based membranes (Meng et al., 2017)

After a fouled membrane is cleaned, pathogen (especially virus) removal efficiency may be temporarily lower than normal, until a cake layer has been sufficiently developed (Chaudhry et al., 2015a), which may take several days or weeks. Branch et al. (2016a) reported that virus removal decreased slightly but became more consistent after the replacement of 10-year-old fouled membranes; they also reported that the removal of *E. coli* and *C. perfringens* increased after the membranes were replaced. Yin et al. (2016) reported that after using pressure relaxation and backwashing to control fouling, the membrane permeability increased and the removal of adenovirus decreased by as much as 0.8 log<sub>10</sub> units; they also reported that the backwash flux had the greatest influence on changes in virus removal efficiency, not the duration of the backwash.

**Feed Water Variations.** Branch et al. (2016b) studied the effects of feed water variation and process failures on the efficiency of the reductions of virus, protozoa and bacteria indicator organisms (FRNA phage, *C. perfringens*, and *E. coli*, respectively). They found that short-term COD loading of 5 g/L temporarily increased virus removal to >6.9 log<sub>10</sub> units (presumably due to a pH drop), short-term NH<sub>3</sub> loading of 0.7 g/L temporarily reduced virus removal to 4.4 log<sub>10</sub> units, and salinity shock of 20 g/L NaCl temporarily reduced virus removal to 3.9 log<sub>10</sub> units. For each of these

three feed water variation cases, virus removal efficiencies recovered or returned to normal within 2 – 3 days after the shock loading event. To address the potential issues of feed water variation, equalization tanks can be used to normalize the quality of feed water, and pretreatment standards for industrial entities can be enforced.

**Process Failures and Membrane Integrity.** Over time, membranes may lose their integrity by experiencing physical damage, pore sizes that are larger than normal, or compromised O-rings or glue lines. Some common reasons for the loss of membrane integrity include manufacturing defects, the use of incompatible chemicals for cleaning, violation of the manufacturer's maximum recommended operating pressure, or damage caused by shear forces or vibrations. Branch et al. (2016b) reported the impact of process failures and membrane integrity on virus removal efficiency, with the following findings:

- Virus removal efficiency decreased immediately from 5.6 log<sub>10</sub> units to 3.5 log<sub>10</sub> units when membranes were compromised (pressure decay rates of 16.5 – 20.0 kPa/min), but quickly recovered within a day
- A 24-hour loss of aeration resulted in *E. coli* and *C. perfringens* removal efficiencies that decreased from >6 log<sub>10</sub> units to 3.8 log<sub>10</sub> units (but returned to normal within a day after aeration returned)
- Immediately following biomass washout, virus removal efficiency decreased from 5.6 log<sub>10</sub> units to 4.0 log<sub>10</sub> units, but recovered within a few days.

To ensure more consistent pathogen removal by the membrane, regular selectivity tests and non-continuous direct monitoring of membrane integrity via pressure decay testing is recommended, in addition to continuous indirect online monitoring of membrane integrity (e.g., permeate water quality, turbidity, TOC) (Sharvelle et al., 2017). For selectivity tests, glass beads of a particular diameter are added to the membrane to determine the percent that pass through. Pressure decay testing is one of the most common tests used in the field to test for defects in the membrane, and can be performed in situ by applying air pressure to the membrane.

**Other Operational Factors.** Operational factors such as the solids retention time and the hydraulic retention time are assumed to have the same effect on pathogen reduction in MBR systems as they do for pathogen reduction during the biological part of similar activated sludge systems (Sidhu et al., 2015) (see Activated Sludge Chapter).

## 5.0 Summary of Data on Pathogen Reduction in Membrane Bioreactors

Overall mean and standard deviation of the log<sub>10</sub> reduction values in MBR systems for different pathogen and indicator organisms are summarized in Table 3. Most of the data reported in the literature on pathogen reduction in MBR systems is for enteric viruses, bacteriophages, and fecal indicator bacteria. A few studies also reported log<sub>10</sub> reductions of *C. perfringens* spores, a proposed indicator

for protozoan pathogens (Branch et al., 2016b; Ottoson et al., 2006; Marti et al., 2011), and only one study reported the removal of actual protozoan pathogens (Ottoson et al., 2006). Likewise, only one study was found to report  $\log_{10}$

reduction values for opportunistic and other bacterial pathogens, including *Legionella* and *Arcobacter* (Harb and Hong, 2017).

**Table 3. Summary of results from literature review on pathogen reduction from wastewater treated in membrane bioreactors.**

Mean $\log_{10}$ reduction values for pathogens (SD = standard deviations)				Mean $\log_{10}$ reduction values for indicators (SD = standard deviations)		
Bacterial Pathogens <sup>a</sup>	Enteric Viruses	Protozoan pathogens	Helminth Eggs	Fecal Indicator Bacteria	<i>C. perfringens</i> spores	Bacteriophages
4.4 (SD: 1.0)	3.3 (SD: 1.2)	>3.9 (Giardia) >1.4 (Crypto)	Not detected in 1 L permeate samples	6.1 (SD: 0.6)	4.6 (SD: 0.7)	3.4 (SD: 1.5)

Sources: Branch et al., 2016b; Chaudhry et al., 2015a; Chaudhry et al., 2015b; De Luca et al., 2013; Franczy et al., 2012; Hai et al., 2014; Harb and Hong, 2017; Kuo et al., 2010; Lv et al., 2006; Marti et al., 2011; Ottoson et al., 2006; Purnell et al., 2016; Shang et al., 2005; Simmons et al., 2011; Wu et al., 2010; Zanetti et al., 2010; Zhang and Farahbakhsh, 2007; Zheng and Liu, 2006; authors' own unpublished data; <sup>a</sup>**Note:** that the only data found for bacterial pathogens were for opportunistic pathogens; the authors of this study (Harb and Hong 2017) used unique methods to detect and quantify these bacterial groups (next generation sequencing with copy-number-adjusted 16S rRNA gene relative abundances multiplied by total bacterial cell counts measured as rpoB gene copy numbers)

Table 3 reports the overall mean (average)  $\log_{10}$  reduction values and standard deviations. However, the median values were very similar, indicating that the data did not have much skew. Also, the values reported in Table 3 are likely underestimates of the true reduction values for two reasons. First, in some of the studies cited in Table 3, the pathogen concentrations were reduced to below the detection limit, but not enough information was provided to treat the dataset using censored statistical models. Second, some authors reported  $\log_{10}$  reductions over the entire system (including reduction due to predation in the aeration tank and by the membrane) while other authors reported  $\log_{10}$  reductions by the membrane only. Given that the removal by the membrane has been shown to be 4 to 7  $\log_{10}$  units greater than the removal in the mixed liquor (Branch et al., 2016b), the distinction between overall reduction and reduction by the membrane only is minimal.

Fecal indicator bacteria removal is consistently high in these systems. For example, the Seine Morée MBR system in France consistently records 7  $\log_{10}$  reductions of *E. coli*. The review of the scientific literature revealed average *E. coli* and enterococci removal values of 6.1  $\log_{10}$  units in MBR systems. However, the systems are less efficient at reducing the concentration of viruses. The average  $\log_{10}$  reduction of enteric viruses and bacteriophages in MBRs was found to be 3.3 and 3.4, respectively. Sano et al., (2016) conducted a meta-analysis of virus reduction in MBR systems, and reported that the reduction of norovirus GII was 3.35  $\log_{10}$  units (95% confidence interval: 2.39, 4.30), and that the reduction of enteroviruses was 2.71  $\log_{10}$  units

(95% confidence interval: 1.52, 3.89). These values are similar to those shown in Table 3. Metagenomics analysis of DNA phages before and after an MBR indicated that no single group of DNA viruses is removed better than others (O'Brien et al., 2017). The summary statistics shown in Table 3 are compiled from values reported in the literature for systems with membranes that have a range of different pore sizes. Ultrafiltration membranes (pore sizes of 0.04  $\mu\text{m}$ ) have been shown to provide significantly different levels of virus reduction than microfiltration membranes (pore sizes of 0.45  $\mu\text{m}$ ) (Yin et al., 2015).

## 6.0 Summary of Data on Pathogens in Membrane Bioreactor Sludge

Membrane bioreactors produce secondary sludge at a rate that depends on the type of influent and the organic load applied. At SIAAP's Seine Morée treatment plant in Paris, which receives domestic wastewater, 0.6 to 0.8 kg of sludge (as TSS) are produced for every kg  $\text{BOD}_5$  removed, and the concentration of sludge in the membrane tank generally varies between 6,000 and 12,000 mg/L VSS. Sludge must be removed daily from MBR systems to maintain proper operating conditions. Prior to disposal or reuse, the sludge must be treated (see chapter on Sludge Management). The concentration of pathogens in secondary sludge from MBR systems has not been well-studied. Nevertheless, more pathogens are caught on the membrane or in the cake layer of an MBR system compared to conventional activated sludge, where some of the pathogens (especially viruses) will be released in the clarified effluent. As such, secondary sludge from MBR

systems will likely have with greater concentrations of pathogens compared to conventional activated sludge systems (Holbrook et al., 2005). Available data for viruses in sludge from MBR systems are shown in Table 4. Because

of the high concentrations of pathogens in MBR secondary sludge, this sludge must be treated for additional pathogen reduction prior to being reused or returned to the environment (see Chapter on Sludge Management).

**Table 4. Concentrations of viruses in membrane bioreactor (secondary) sludge.**

Viral Pathogen	Concentration (/g dry sludge)
Adenovirus (qPCR)	1.3E+4 copies
Culturable enteric virus	2.1E+2 MPN
Enterovirus (RT-qPCR)	7.9E+2 copies
Norovirus GI (RT-qPCR)	2.0E+4 copies
Norovirus GII (RT-qPCR)	1.6E+5 copies

Reference: Simmons & Xagorarakis (2011); RT-qPCR = detected using qPCR with reverse transcription

## 7.0 Conclusions

Among the different types of secondary wastewater treatment technologies, MBR systems provide very efficient pathogen reductions if they are maintained appropriately. Virus removal is the least efficient of all pathogen types. However, MBR systems can be designed and operated in a way that enhances virus removal efficiency as much as possible by using ultrafiltration membranes, monitoring the development of fouling, and conducting periodic selectivity and integrity testing. Fecal indicator bacteria should not be used as a surrogate for enteric virus removal in MBR systems, but bacteriophages appear to be an appropriate indicator for enteric virus removal in these systems. There are not enough data on the reduction of protozoan pathogens in MBR systems, but the reduction of *C.*

*perfringens* spores is greater than that of viruses and bacteriophages but less than that of fecal indicator bacteria.

The efficiency of pathogen reduction in MBR systems will undoubtedly be influenced by local conditions, including the quality and composition of incoming untreated wastewater, operation and maintenance activities, troubleshooting capabilities, as well as socioeconomic and geographical factors that affect a community's ability to provide proper maintenance. MBR is a complex treatment process that should be intensively validated before assigning  $\log_{10}$  reduction credits. Any  $\log_{10}$  reduction values reported herein should require local validation prior to being used for regulatory purposes.

## References

- Chaudhry, R.M., Nelson, K.L. and Drewes, J.E. (2015). Mechanisms of pathogenic virus removal in a full-scale membrane bioreactor. *Environmental Science and Technology*. 49, pp. 2815-2822.
- Bosch, A., Pinto, R.M. and Abad, F.X. (2006). Survival and transport of enteric viruses in the environment. *Viruses in Foods*. pp. 151-187.
- Branch, A., James, G., Trinh, T., Ta, T.M., Leslie, G. and Le-Clech, P. (2016). Membrane ageing and replacement-Impact on pathogen removal in full-scale MBR. *Proceedings of the American Water Works Association Annual Water Quality Technology Conference and Exposition*. pp. 13-17.
- Branch, A., Trinh, T., Carvajal, G., Leslie, G., Coleman, H.M., Stuetz, R.M. *et al.* (2016). Hazardous events in membrane bioreactors-Part 3: Impacts on microorganism log removal efficiencies. *Journal of Membrane Science*. 497, pp. 514-523.
- Chaudhry, R.M., Holloway, R.W., Cath, T.Y. and Nelson, K.L. (2015). Impact of virus surface characteristics on removal mechanisms within membrane bioreactors. *Water Research*. 84, pp. 144-152.
- Chiemchaisri, C., Yamamoto, K. and Vigneswaran, S. (1993). Household membrane bioreactor in domestic wastewater treatment. *Water Science and Technology*. 27, pp. 171-178.
- De Luca, G., Sacchetti, R., Leoni, E. and Zanetti, F. (2013). Removal of indicator bacteriophages from municipal wastewater by a full-scale membrane bioreactor and a conventional activated sludge process: Implications to water reuse. *Bioresource Technology*. 129, pp. 526-531.
- Francy, D.S., Stelzer, E.A., Bushon, R.N., Brady, A.M.G., Williston, A.G., Riddell, K.R. *et al.* (2012). Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters. *Water Research*. 46, pp. 4164-4178.
- Gaveau, A., Coetsier, C., Roques, C., Bacchin, P., Dague, E. and Causserand, C. (2017). Bacteria transfer by deformation through microfiltration membrane. *Journal of Membrane Science*. 523, pp. 446-455.
- George, I., Crop, P. and Servais, P. (2002). Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods. *Water Research*. 36, pp. 2607-2617.
- Guo, W., Ngo, H.H. and Li, J. (2012). A mini-review on membrane fouling. *Bioresource Technology*. 122, pp. 27-34.
- Hai, F.I., Riley, T., Shawkat, S., Magram, S.F. and Yamamoto, K. (2014). Removal of pathogens by membrane bioreactors: a review of the mechanisms, influencing factors and reduction in chemical disinfectant dosing. *Water*. 6, pp. 3603-3630.
- Harb, M. and Hong, P.Y. (2017). Molecular-based detection of potentially pathogenic bacteria in membrane bioreactor (MBR) systems treating municipal wastewater: a case study. *Environmental Science and Pollution Research*. 24, pp. 5370-5380.
- Holbrook, R.D., Massie, K.A. and Novak, J.T. (2005). A comparison of membrane bioreactor and conventional-activated-

sludge mixed liquor and biosolids characteristics. *Water Environment Research*. 77, pp. 323-330.

Kim, T.D. and Unno, H. (1996). The roles of microbes in the removal and inactivation of viruses in a biological wastewater treatment system. *Water Science and Technology*. 33, pp. 243-250.

Kuo, D.H.W., Simmons, F.J., Blair, S., Hart, E., Rose, J.B. and Xagorarakis, I. (2010). Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. *Water Research*. 44, pp. 1520-1530.

Lv, W., Zheng, X., Yang, M., Zhang, Y., Liu, Y. and Liu, J. (2006). Virus removal performance and mechanism of a submerged membrane bioreactor. *Process Biochemistry*. 41, pp. 299-304.

Marti, E., Monclús, H., Jofre, J., Rodríguez-Roda, I., Comas, J. and Balcazar, J.Luis (2011). Removal of microbial indicators from municipal wastewater by a membrane bioreactor (MBR). *Bioresource Technology*. 102, pp. 5004-5009.

Matz, C., McDougald, D., Moreno, A.M., Yung, P.Y., Yildiz, F.H. and Kjelleberg, S. (2005). Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *Proceedings of the National Academy of Sciences of the United States of America*. 102, pp. 16819-16824.

Meng, F., Zhang, S., Oh, Y., Zhou, Z., Shin, H.S. and Chae, S.R. (2017). Fouling in membrane bioreactors: an updated review. *Water Research*. 114, pp. 151-180.

O'Brien, E., Munir, M., Marsh, T., Heran, M., Lesage, G., Tarabara, V.V. *et al.* (2017). Diversity of DNA viruses in effluents of membrane bioreactors in Traverse City, MI (USA) and La Grande Motte (France). *Water Research*. 111, pp. 338-345.

Ottoson, J., Hansen, A., Björlenius, B., Norder, H. and Stenström, T.A. (2006). Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. *Water Research*. 40, pp. 1449-1457.

Purnell, S., Ebdon, J., Buck, A., Tupper, M. and Taylor, H. (2016). Removal of phages and viral pathogens in a full-scale MBR: Implications for wastewater reuse and potable water. *Water Research*. 100, pp. 20-27.

Sano, D., Amarasiri, M., Hata, A., Watanabe, T. and Katayama, H. (2016). Risk management of viral infectious diseases in wastewater reclamation and reuse: Review. *Environment International*. 91, pp. 220-229.

Schmitz, B., Kitajima, M., Campillo, M., Gerba, C. and Pepper, I. (2016). Virus reduction during advanced Bardenpho and conventional wastewater treatment processes. *Environmental Science and Technology*. 50(17), pp. 9524-9532.

Shang, C., Wong, H.M. and Chen, G. (2005). Bacteriophage MS-2 removal by submerged membrane bioreactor. *Water Research*. 39, pp. 4211-4219.

Sharvelle, S., Ashbolt, N., Clerico, E., Hultquist, R., Leverenz, H. and Olivieri, A. (2017). Risk-Based Framework for the Development of Public Health Guidance for Decentralized Non-Potable Water Systems. *Proceedings of the Water Environment Federation*. 2017, pp. 3799-3809.

Sidhu, J.P.S., Ahmed, W., Hodggers, L., Smith, K., Palmer, A., Wylie, J. *et al.* (2015). Development of Validation Protocol for

Activated Sludge Process in Water Recycling. Australian Water Recycling Centre of Excellence.

Simmons, F.J., Kuo, D.H.W. and Xagorarakis, I. (2011). Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. *Water Research*. 45, pp. 2739-2750.

Stevik, T.K., Aa, K., Ausland, G. and Hanssen, J.F. (2004). Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. *Water Research*. 38, pp. 1355-1367.

Wang, Z., Ma, J., Tang, C.Y., Kimura, K., Wang, Q. and Han, X. (2014). Membrane cleaning in membrane bioreactors: a review. *Journal of Membrane Science*. 468, pp. 276-307.

Wu, J., Li, H. and Huang, X. (2010). Indigenous somatic coliphage removal from a real municipal wastewater by a submerged membrane bioreactor. *Water Research*. 44, pp. 1853-1862.

Yin, Z., Tarabara, V.V. and Xagorarakis, I. (2016). Effect of pressure relaxation and membrane backwash on adenovirus removal in a membrane bioreactor. *Water Research*. 88, pp. 750-757.

Yin, Z., Tarabara, V.V. and Xagorarakis, I. (2015). Human adenovirus removal by hollow fiber membranes: Effect of membrane fouling by suspended and dissolved matter. *Journal of Membrane Science*. 482, pp. 120-127.

Zanetti, F., De Luca, G. and Sacchetti, R. (2010). Performance of a full-scale membrane bioreactor system in treating municipal wastewater for reuse purposes. *Bioresource technology*. 101, pp. 3768-3771.

Zhang, K. and Farahbakhsh, K. (2007). Removal of native coliphages and coliform bacteria from municipal wastewater by various wastewater treatment processes: implications to water reuse. *Water Research*. 41, pp. 2816-2824.

Zheng, X. and Liu, J.X. (2006). Mechanism investigation of virus removal in a membrane bioreactor. *Water Science and Technology: Water Supply*. 6, pp. 51-59.