**Effect of pressure relaxation and membrane backwash on adenovirus removal in
a membrane bioreactor**

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by

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Abstract

Pressure relaxation and permeate backwash are two commonly used physical methods for membrane fouling mitigation in membrane bioreactor (MBR) systems. In order to assess the impact of these methods on virus removal by MBRs, experiments were conducted in a bench-scale submerged MBR treating synthetic wastewater. The membranes employed were hollow fibers with the nominal pore size of 0.45 μm . The experimental variables included durations of the filtration ($t_{TMP>0}$), pressure relaxation ($t_{TMP=0}$) and backwash ($t_{TMP<0}$) steps. Both pressure relaxation and permeate backwash led to significant reductions in removal of human adenovirus (HAdV). For the same value of $t_{TMP>0}/t_{TMP=0}$, longer filtration/relaxation cycles (i.e. larger $t_{TMP} + t_{TMP=0}$) led to higher transmembrane pressure (TMP) but did not have a significant impact on HAdV removal. A shorter backwash ($t_{TMP<0} = 10$ min) at a higher flow rate ($Q = 40$ mL/min) resulted in more substantial decreases in TMP and HAdV removal than a longer backwash ($t_{TMP<0} = 20$ min) at a lower flow rate ($Q = 20$ mL/min) even though the backwash volume ($Qt_{TMP<0}$) was the same. HAdV removal returned to pre-cleaning levels within 16 h after backwash was applied. Moderate to strong correlations ($R^2 = 0.63$ to 0.94) were found between TMP and HAdV removal.

Keywords: membrane bioreactor, membrane fouling, human adenovirus, pressure relaxation, backwash

1. Introduction

Membrane bioreactors, a combination of activated sludge process and membrane filtration, have developed into a staple technology for municipal and industrial wastewater treatment and a particularly attractive treatment choice for water reuse (Judd 2010)(Hoinkis et al. 2012, Andrade et al. 2014, Yin and Xagorarakis 2015). Compared to conventional activated sludge wastewater treatment systems, MBRs are more compact and, generally, afford more stable performance (Choi et al. 2002). With proper design and optimized operational conditions MBRs can remove a wide range of pollutants (Vaid et al. 1991, Pankhania et al. 1994, Beaubien et al. 1996, Kishino et al. 1996, Gujer et al. 1999, Van der Roest et al. 2005)(Phan et al. 2014, Sun et al. 2015, Sun et al. 2013, Boonnorat et al. 2014, Malaeb et al. 2013).

Membrane fouling in MBRs remains a major technical challenge (Bouhabila et al. 2001, Judd 2008, Cornel and Krause 2008). During MBR operation, biosolids as well as colloidal and macromolecular species may deposit and accumulate on membrane surfaces resulting in a decline in permeate flux. A number of membrane fouling mitigation methods have been developed including pressure relaxation, air sparging and membrane cleaning by hydraulic or chemical means. Hydraulically reversible fouling is defined as fouling that can be removed by a hydraulic wash, while hydraulically irreversible fouling refers may only be removed by chemical cleaning (Chang et al. 2002) and is typically due to intrapore fouling. Air sparging mainly targets external fouling, such as a loosely attached cake layer on membrane surface

while backwash can also remove internal fouling (Bouhabila et al. 2001, Psoch and Schiewer 2006).

Air sparging is very commonly applied, especially in submerged aerobic MBRs with ultrafiltration and microfiltration membranes, where aeration serves a dual purpose of providing oxygen to bacteria and mitigating membrane fouling. Coarse air bubbles create shear at membrane surfaces, and partially remove loosely attached fouling layers. It has been well documented that air sparging can enhance hydraulic permeability of MBR membranes with strong positive correlations found between air sparging rate and fouling reduction (Chang and Judd 2002, Yu et al. 2003, Ghosh 2006, Fan and Zhou 2007, Delgado et al. 2008). To further reduce membrane fouling, air sparging is often coupled with pressure relaxation. (Hong et al. 2002) clearly demonstrated that permeate flux decreased slower when periodical pressure relaxation was applied. (Wu et al. 2008) reached a qualitatively similar conclusion reporting that the extent of fouling was related to the duration and frequency of pressure relaxation.

Membrane backwash is another method that is widely used to reduce membrane fouling in MBRs. (Hwang et al. 2009) suggested that backwash by deionized water can completely remove membrane cake and alleviate intrapore fouling. (Yigit et al. 2009) reported that membrane resistance was reduced ~160% after backwash and concluded that backwash effectively diminished reversible fouling due to pore blocking and cake layer formation. Backwash parameters such as duration, interval

and backwash flow rate can significantly affect fouling (Wu et al. 2008, Hwang et al. 2009). (Delgado et al. 2008) reported that backwash time had a strong impact on residual fouling. (Kim and DiGiano 2006) showed that higher backwash frequency could reduce long-term fouling rate. With the same backwash volume, higher backwash flux was more effective in fouling reduction than a longer duration of the backwash (Zsirai et al. 2012).

Enteric viruses, as a type of infectious pathogens in wastewater, pose a significant threat to public safety. Most published studies on virus removal by MBRs focused on *bench- and pilot-scale* MBR systems and bacteriophages such as MS2, T4 and F-specific and somatic coliphage (Cicek et al. 1998, Hu et al. 2003, Shang et al. 2005, Comerton et al. 2005, Fiksdal and Leiknes 2006, Lv et al. 2006, Zhang and Farahbakhsh 2007, Zheng and Liu 2007, Tam et al. 2007, Ravindran et al. 2009, Hirani et al. 2010, Chaudhry et al. 2015a, Fox and Stuckey 2015, Hmaied et al. 2015, van den Akker et al. 2014). Two bench-scale studies employed human viruses; (Madaeni et al. 1995) reported that the removal of poliovirus ranged from 1.3 to 1.8 logs while (Ottoson et al. 2006) showed that the log reduction value (LRV) for enterovirus and norovirus ranged from 0.5 to 1.8 logs.

To our knowledge, there have been only seven studies on the removal of viruses in *full-scale* systems. Norovirus removal in a full-scale MBR utilities was reported to cover a very wide range from 0 (i. e. no removal) to 5.5 logs (da Silva et al. 2007)

LRVs of ~ 5.1 logs for enteroviruses, 3.9 logs for norovirus, and 5.5 logs for adenoviruses were reported (Kuo et al. 2010, Simmons et al. 2011, Simmons and Xagorarakis 2011). (Zanetti et al. 2010) measured LRVs for F-specific coliphage and somatic coliphage to be 6 logs and 4 logs, respectively. Two recent studies investigated virus removal in full-scale MBRs equipped with 0.04 μm membranes: the reductions of adenovirus, norovirus and F+ coliphage were 3.9 – 5.5 logs, 4.6 - 5.7 logs and 5.4 – 7.1 logs, respectively after MBR treatment (Chaudhry et al. 2015b), while removals of somatic, F-RNA, GB124, MS2 and B14 coliphages were 5.3 logs, 3.5 logs, 3.8 logs, 2.2 logs and 2.3 logs. All the studies indicated that MBR systems were not able to serve as an absolute barrier against viruses, even though high virus removals were achieved in many cases.

The role of biofilm in virus removal by MBRs has been studied by (Wu et al. 2010) who found that the clean membrane ($d_{pore} = 0.4 \mu\text{m}$) contributed only ~0.5 logs removal of somatic coliphages; in contrast, when covered with a biofilm the same membrane could remove 1.8 to 2.6 logs of the virus. Similarly, (Shang et al. 2005) observed that an MBR with the nominal pore size of 0.4 μm could initially (i.e. prior to significant membrane fouling) only remove 0.4 logs of MS-2 coliphage. After 21 days of operation, the removal efficiency increased to 2.3 logs; it was concluded that membrane biofilm played an important role in removing the virus. Despite the fact that one or several fouling mitigation methods are routinely applied in MBR plants, little is known about the impact that these practices have on virus removal (Table 1).

Most of the published work on the subject focused on chemical cleaning and employed bacteriophages.

Table 1 – Effect of membrane fouling mitigation methods on virus removal in submerged MBRs

MBR	d_{pore}	Fouling mitigation method	Effect on fouling	Virus	Effect on virus removal	Reference
Pilot scale	0.4 μm	Chemical clean	NA	F-specific coliphage	Concentration in the effluent increased from 0.5 to 18.5 PFU/100 mL	(Tam et al. 2007)
	0.1 μm	Chemical clean	NA	MS -2 coliphage	Concentration in the effluent increased by up to 32 PFU/100 mL	(Hirani et al. 2014)
Bench scale	0.4 μm	Chemical backwash	NA	Somatic coliphage	$\Delta LRV \leq 1^*$	(Wu et al. 2010)
	0.4 μm	Chemical backwash	Original pressure recovered	MS -2 coliphage	$0.4 < \Delta LRV \leq 2.5$	(Shang et al. 2005)
	0.1 μm and 0.22 μm	Tap water flush	Hydraulic resistance decreased from $6.4 \cdot 10^{12}$ to $3.5 \cdot 10^{12} \text{ m}^{-1}$	T4 coliphage	Concentration in the effluent increased from <2 to 400 - 500 PFU/mL	(Zheng et al. 2005)
		Chemical clean	Hydraulic resistance further decreased from $3.5 \cdot 10^{12}$ to $1.4 \cdot 10^{12} \text{ m}^{-1}$		Concentration in the effluent further increased to 2000 - 16000 PFU/mL	
	0.1 μm	Tap water flush	Hydraulic resistance decreased from $2.1 \cdot 10^{13}$ to $8 \cdot 10^{12} \text{ m}^{-1}$	T4 coliphage	LRV decreased from 6 logs to 5 logs	(Lv et al. 2006)
		Chemical clean	Hydraulic resistance further decreased to $4.1 \cdot 10^{12} \text{ m}^{-1}$		LRV further decreased to 4 logs	
	0.22 μm	Tap water flush	Hydraulic resistance decreased from $6.3 \cdot 10^{12}$ to $3.5 \cdot 10^{12} \text{ m}^{-1}$		LRV decreased from 5 logs to 3.3 logs	
		Chemical clean	Hydraulic resistance further decreased to $1.5 \cdot 10^{12} \text{ m}^{-1}$		LRV further decreased to 1.9 logs	
Full scale	0.4 μm	Air sparging	Fouling reduced with increasing sparging rate	MS -2 coliphage	Virus rejection enhanced with increasing sparging rates	(Fox and Stuckey 2014)
				T4 coliphage		

* ΔLRV : LRV reduction

It has been reported that chemical cleaning that completely removed the membrane biofilm greatly affected the removal of viruses and it could take more than 24 h for

the removal to recover to pre-cleaning levels (Lv et al. 2006, Tam et al. 2007). Only two studies (Lv et al. 2006, Zheng et al. 2005) evaluated the effect of hydraulic flushing (not backwash) by cleaning the membrane surface with tap water, using the same bench scale MBR system and T4 coliphage.

One recently published paper (Fox and Stuckey 2014) evaluated the impact of air sparging on virus removal in an anaerobic MBR, and indicated that higher sparging rates led to greater removal of MS-2 and T4 coliphage. To our knowledge, the impacts of pressure relaxation and permeate backwash on virus removal in MBR systems have not been investigated. The effect of these fouling mitigation methods on the removal of human adenovirus 40 (HAdV 40), an infectious enteric virus is at the focus on the present work.

2. Materials and Methods

2.1. Cell culture experiment and virus incubation

A549 cell line has been suggested as an efficient cell line for HAdV-40 (ATCC, VR 931) (Witt and Bousquet 1988, Lee et al. 2004) and it was used to grow HAdV in this study. Details of virus incubation were described in (Yin et al. 2015).

2.2. Membrane preparation

The polyvinylidene fluoride (PVDF) hollow fiber membrane used in this work had the nominal pore size of 0.45 μm and the outer diameter of 1.3 mm. Membrane units

were made by looping and potting 14 hollow fiber segments (effective length: 70 cm each) in a short (~10 cm) piece of 1/2" ID PTFE tubing with an adhesive (Loctite). Each membrane unit had an effective surface area of ~400 cm², and 4 such units were used in each experiment giving a total effective surface area of ~1600 cm². Prior to each test, membrane was soaked in deionized (DI) water for at least 24 h, and then compacted by filtering DI water for 12 h.

2.3. Bench-scale submerged MBR

A schematic of the bench-scale MBR system is shown in Figure 1. The MBR could accommodate 25 L of activated sludge and the working volume was 20 L. A peristaltic digital pump (model 07523-80, MasterFlex L/S) served as the permeate pump. The system was running in a constant flux regime ($Q = 31.3$ mL/min; $j = 3.26 \cdot 10^{-6}$ m/s). Transmembrane pressure (TMP) and permeate flow rate were measured by a digital pressure sensor (Cole-Parmer, 68075-00) and digital flow meter (model 106-4-C-T4-C10, McMillan), respectively. A LabView program was developed to (1) maintain the constant permeate flow using a proportional-integral-derivative (PID) algorithm; (2) conduct periodical pressure relaxation by turning the permeate pump on and off; (3) record data from the flow meter and the pressure sensor.

Activated sludge from East Lansing wastewater treatment plant was incubated in a 25 L glass cylinder tank with synthetic wastewater (Table 2) for over three months.

Membranes were then placed in the activated sludge and the MBR system was run for over three months. The hydraulic retention time (HRT) was 0.5 day, and mixed liquor suspended solids (MLSS) concentration was kept at 4.5 g/L based on daily MLSS measurements. Aeration was continuously applied throughout the experiment at the rate of 0.57 m³/h. A preliminary test indicated that the MBR was able to remove ~97% of total organic carbon (data not shown).

Figure 1: Schematic of the submerged MBR

Table 2: Composition of the synthetic wastewater *

Chemicals	Daily dose (g)	Chemicals	Daily dose (mg)
Glucose	15	H ₃ BO ₃	3.60
Peptone	5	CuSO ₄ · 5H ₂ O	0.72
KH ₂ PO ₄	1.6	KI	5.12
(NH ₄) ₂ SO ₄	5.2	MnCl ₂ · 4H ₂ O	2.88
MgSO ₄ · 7H ₂ O	3.2	NaMoO ₄ · 2H ₂ O	1.44
CaCl ₂ · 2H ₂ O	1.6	ZnSO ₄ · 7H ₂ O	2.88
EDTA	0.24	CoCl ₂ · 6H ₂ O	3.60
NaCl	5	FeCl ₃ · 6H ₂ O	36.00

* The pH of the synthetic wastewater was adjusted to 7.5 before use. The mineral makeup of the synthetic wastewater was adapted from (Yuan et al. 2009) and (Broughton et al. 2008).

2.4. Fouling and backwash experiments

A total of three virus removal experiments were conducted. Each experiment consisted of a 2-hour water filtration stage (conditioning stage), an 8-day fouling stage (Stage 1) and two 2-day backwash stages (Stage 2 and 3). Periodical pressure relaxation was applied during fouling and backwash stages with the formats described in Table 3. All samples were stored at -80 °C until analysis.

Table 3 – Parameters of pressure relaxation and backwash

Exp. #.	Pressure relaxation ($TMP = 0$)	Permeate backwash ($TMP < 0$)	
	$t_{TMP>0} / t_{TMP=0}$	Backwash flux	$t_{TMP<0}$
1	25 min / 5 min	$4.2 \cdot 10^{-6}$ m/s	10 min
2	25 min / 5min	$2.1 \cdot 10^{-6}$ m/s	20 min
3	50 min / 10 min	$4.2 \cdot 10^{-6}$ m/s	10 min

Conditioning stage (duration = 2 h): A set of pristine membranes was placed in a tank with 20 L of DI water and air diffuser on the bottom. Virus was added into the tank and mixed for 1 h by aeration. Membrane filtration was carried out at a constant flow rate of 31.3 mL/min for 1 h. Feed and permeate samples were collected at the end of the stage.

Stage 1 (duration = 8 d): The membrane was installed in the MBR system and was then operated as described in section 2.3. Two sets of feed and permeate samples were collected each day. For sampling, 40 mL of virus stock solution was spiked into the activated sludge ~ 6 min before pressure relaxation. The first set of samples was taken 50 s before pressure relaxation, while the second set of samples was collected 3 min (when flow rate was constant) after pressure relaxation. Feed samples with activated

sludge were settled for 15 min, and then passed through 0.22 µm Millipore filters. The virus concentration in the filtrate (assumed to represent the liquid phase of the mixed liquor) was considered as the feed concentration of the virus.

Stages 2 and 3 (duration = 2 d each): At the beginning of each of these stages, the membrane was backwashed using permeate water following the format described in Table 6. Two sets of samples were collected every 16 h, following the same sampling strategy as in Stage 1. 40 mL of virus stock solution was added into the activated sludge before each sampling point as in Stage 1.

2.5. DNA extraction and quantitative polymerase chain reaction (qPCR)

Virus DNA in each sample was extracted by using MagNa Pure Compact System with Nucleic Acid Isolation Kits (Roche Applied Sciences), following the manufacture's instruction manual. Carrier RNA (Qiagen) was used to increase DNA recovery. The DNA extracts were stored at -80 °C immediately after extraction. Following DNA extraction, virus quantification was conducted using qPCR (Roche Light Cycler). Sequences of primers and TaqMan probe were adopted from (Heim et al. 2003). The sequence (5'-3') of forward primer, reverse primer and probe are GCCACGGTGGGGTTTCTAACTT, GCCCCAGTGGTCTTACATGCACATC, and FAM-TGCACCAGACCCGGGCTCAGGTACTCCGA-TAMRA, respectively. HAdV concentration was calculated based on crossing point C_p values generated by LightCycler software and a previously developed standard curve.

2.6. Inhibition test of qPCR

Polymerase chain reaction may be inhibited in the presence of organic matter (Sutlović et al. 2005). In order to rule out the potential inhibition on qPCR by organic matter in the activated sludge, an inhibition test was conducted: 1 mL of HAdV stock solution was added into 9 mL of DI water and 9 mL of feed sample. DNA extraction and qPCR were carried out accordingly. No significant difference of measured virus concentration between DI water and feed sample was found.

3. Results and Discussion

3.1. Membrane fouling and transmembrane pressure buildup

Figure 2 summarizes *TMP* profiles in all three experiments. The duration of the filtration period in each cycle in exp. 1 and exp. 2 was 25 min, while in exp. 3 it was 50 min (Table 3). The LabView program was used to log in *TMP* data every second. Each dot in Figure 2 represents the average value of *TMP* from the 3rd min (when the flow rate becomes constant) to the end of filtration period in each cycle. In exp. 1 and exp. 2, which were performed in the 25 min/5 min filtration/relaxation cycles, *TMP* increased from ~ 3.6 kPa to 11 kPa during 8 days of the fouling test. In exp. 3 (performed in 50 min /10 min filtration relaxation cycles) *TMP* increased from ~ 4.0 kPa to 15 kPa over the same period. The results suggest that less frequent cycling leads to more fouling even though the ratio of relaxation time ($t_{TMP=0}$) to filtration time ($t_{TMP>0}$) is maintained the same. One explanation for this trend is that fouling accumulates over the entire filtration stage of the cycle while the capability of air

sparging to remove fouling during the relaxation stage is limited so that only the most recently (less than 50 min in our experimental conditions) formed layer can be removed by aeration. Similar results were reported by (Wen et al. 1999), with more fouling observed in an MBR with an operational mode of 8 min filtration / 2 min relaxation compared to 4 min filtration/ 1 min relaxation. They also observed that the 2 min/ 0.5 min format resulted in more fouling than the 4 min on/ 1 min off format. Wu et al. (2008) found the 220 s / 20 s off format created more fouling than the 440 s / 40 s. This is probably because $t_{TMP=0}$ of 20 to 30 s is insufficient for air sparging to remove all reversible fouling.

The data also show how the backwash flow rate and duration affect TMP . In exp.1, backwash was conducted at the flux of $4.2 \cdot 10^{-6}$ m/s for 10 min, and the TMP decreased by 3.6 kPa and 2.5 kPa in Stage 2 and 3, respectively. In exp. 2 with 20 min backwash at $2.1 \cdot 10^{-6}$ m/s, the TMP decreased by 2.6 kPa and 1.6 kPa in Stage 2 and 3, respectively. With the same backwash flow rate and duration, TMP in exp. 3 dropped by 3.5 kPa at Stage 2, which is similar to what was observed in exp. 1. However, TMP only decreased by 1.0 kPa in Stage 3. These data indicate that with a given backwash volume, backwash flux is more effective than backwash duration in controlling TMP . This is consistent with results reported by (Zsirai et al. 2012), who made a similar conclusion based on their results with a pilot-scale submerged MBR. Moreover, the data also show that the effect of backwash on TMP was weaker when the membrane was subject to the 2nd backwash compared to the 1st backwash, and this

tendency seems to be enhanced with a longer duration of the filtration/relaxation cycle (exp. 3).

Figure 2 – Transmembrane pressure as a function of filtration time and the effect of backwash.

3.2. Human adenovirus removal

The removal of HAdV 40 from DI water in experiments 1, 2 and 3 was 1.22, 1.07 and 1.07 logs respectively. When the membrane units were placed in activated sludge and the filtration was conducted at the same flow rate, LRV increased to ~ 2 logs in all three experiments (Figure 3). This conflicts with the data presented by (Shang et al. 2005) where the initial LRV from activated sludge was almost the same (~ 0.3 log) as the removal from DI water. This is because the first sample in our experiments was

collected when filtration had been carried out for ~ 25 min (exp. 1 and exp. 2) or 50 min (exp. 3), and membrane fouling occurred during that time. More importantly, even though the membrane used by (Shang et al. 2005) had a pore size (0.4 μm) similar to that of the membrane employed in this work, the virus used in their study (MS-2 phage) was much smaller (20 - 25 nm, (Shang et al. 2005)) than the human adenovirus 40 (70 -140 nm, (Xagorarakis et al. 2014)). The pore blockage effect on the removal of MS-2 phage is not as significant as on the removal of HAdV 40, since it is easier for smaller viruses to pass through partially blocked pores.

In exp. 1, LRV increased from 2.33 logs (before relaxation) and 2.06 logs (after relaxation) at the beginning, to 3.87 logs and 2.78 logs at day 4 respectively. Then LRV remained at the approximately same level for the last 4 days. In exp. 2, the observed LRV started at ~2 logs, increased to 4.19 logs (before relaxation) and 3.54 logs (after relaxation) at day 4, and ended at 4.70 logs and 3.67 logs at day 8. Thus virus removal increased much faster during the first 4 days compared to last 4 days.

A similar trend was observed in previous studies. In the 20-day experiment carried out by (Shang et al. 2005), the LRV of MS-2 grew from 0.3 logs to 2.3 logs in the first 10 days, then reached 2.5 logs at the 20th day. (Madaeni et al. 1995) implemented a 6-h experiment with 0.22 μm PVDF membranes, in which the increase of poliovirus rejection was rapid between 0.5 h and 2 h, and slowed down afterward. Such removal profile has also been reported for chemicals. Virus removal in exp. 3 in our study

increased steadily throughout Stage 1. A larger number of samples taken closer to the end of Stage 1 would be needed to further investigate the trend.

In experiment 1 and 3, a backwash was applied for 10 min at the flux of $4.2 \cdot 10^{-6}$ m/s prior to each stage 2 and stage 3. In exp. 1, the LRV before and after pressure relaxation decreased by 0.91 and 0.72 log at the result of the first backwash and by 0.87 and 0.60 log as a result of the second backwash, respectively. In exp. 3, LRV reduced by 0.76 and 0.87 log due to the 1st backwash, while the difference between LRVs before and after the 2nd backwash could not be calculated as virus concentrations in permeate samples at day 10 in Stage 3 were below the detection limit. The same volume of membrane permeate was used in exp. 2 for backwash, but at the flux of $2.1 \cdot 10^{-6}$ m/s for 20 minutes. As a result, backwash 1 lowered LRV by 0.33 and 0.31 log while backwash 2 barely affected the LRV.

A

Figure 3 – Effects of pressure relaxation and backwash on the removal of HAdV 40 in submerged MBR operated under three different filtration / pressure relaxation schedules and backwash protocols (see Table 1):
A: exp. 1; B: exp. 2; C: exp. 3.

Reduction of HAdV removal caused by different backwash formats is summarized in Figure 4, and t-test shows that LRV reduction due to backwash with higher flow rate is significantly greater ($p < 0.05$) than with a longer duration backwash. These data demonstrate that backwash had a similar impact on *TMP* and virus removal: (1) with the same permeate volume used for backwash, higher backwash flux causes a larger reduction LRV than a longer backwash does; (2) Decrease of virus removal appears to be greater during the 1st backwash compared to the 2nd backwash. (Wu et al. 2010) studied the impact of chemical backwash (by a NaClO solution) on virus removal in a full-scale MBR. They found virus rejection by membrane dropped 0 – 1.5 logs after each backwash. In all our experiments, the virus removal recovered to the pre-backwash level within 16 h after the backwash was applied. In contrast, (Tam et al. 2007) investigated the removal of male-specific (F+) coliphage in a pilot-scale MBR system equipped with microfiltration membranes. They observed that it may take more than 24 h for the recovery of virus removal after the fouled membrane is subjected to chemical cleaning.

As shown above, longer filtration/relaxation cycles caused higher *TMP*. However, this effect didn't apply to virus removal. Student's t test shows LRV at neither before relaxation nor after relaxation in Stage 1 of experiment 3 is significantly higher ($p > 0.05$) compared to experiment 1 and 2 combined. Probability plots show that LRVs in all experiments are normally distributed. Moreover, it is notable that in all sampling events, HAdV removal before pressure relaxation is always higher than after pressure

relaxation, and the mean LRV before relaxation was 0.74, 0.48, and 0.42 log higher in Stage 1 of experiment 1, 2 and 3, respectively. This suggests that the portion of fouling that can be reversed by aeration during pressure relaxation enhances virus removal. Our previous study indicates that the reversible fouling caused by silica particles (3.45 μm in diameter) reduced HAdV removal, especially in the case small pore size (0.04 μm) membranes (Yin et al. 2015). This suggests that reversible fouling could either increase or decrease virus removal, and that the property of foulants is the dominant factor in this regard.

As shown in Figure 5, this enhancement in LRV due to reversible fouling appears to be unaffected by the duration of filtration/relaxation cycles, as there was no significant difference ($p > 0.05$) between combined data from exp. 1 and exp. 2 on the one hand and the data from exp. 3 on the other hand. This is probably because over the short term, *TMP* increase is caused by reversible fouling. In each filtration cycle after the flow rate reached a constant level, the change in *TMP* was very slow. This is supported by the t-test which showed the difference of *TMP* before and after pressure relaxation at sampling events in exp. 3 was not significantly ($p > 0.05$) higher compared to exp.1 and exp. 2 combined. Thus *TMP* did not increase further in each filtration cycle despite the fact that duration of filtration cycles was doubled.

Figure 4 – Decrease in HAdV removal as a result of backwash for two different backwash formats.

Figure 5 – Decrease in HAdV removal as a result of pressure relaxation for two different formats of the filtration/relaxation (F/R) cycle.

3.3. Relationship between transmembrane pressure and virus removal

Figure 6 demonstrates the correlation between transmembrane pressure (TMP) and virus removal (LRV). ΔP and LRV in exp. 1 and exp. 2 were moderately correlated ($R^2 = 0.63$ and 0.78 , $p < 0.01$, respectively) to each other, while a relatively strong

402 correlation ($R^2 = 0.94$, $p < 0.01$) was observed in exp. 3. A moderate correlation (R^2
403 $= 0.72$, $p < 0.01$) was obtained when the analysis was applied to data from all three
404 experiments. The correlations found in our study are stronger than those observed by
405 (Wu et al. 2010), who reported a moderate correlation ($R^2 = 0.656$) between *TMP*
406 and LRV of indigenous somatic coliphages. The possible correlation between *TMP*
407 and LRV was also explored by (Shang et al. 2005), but these authors suggested that
408 the correlation only exists when the food to mass ratio is low. In sum, *TMP* may be
409 used to estimate levels of virus removal in MBR systems, and higher *TMP* generally
410 leads to greater virus removal. However, the quantitative correlations may be
411 system-dependent and vary with the virus type.

A

B

Figure 6 – Correlations between HAdV removal and transmembrane pressure in experiments 1 (A), 2 (B) and 3 (C). For each data point, *TMP* represents the pressure averaged over 11 s interval around the corresponding sampling point.

Conclusions

This study explored how two fouling mitigation methods - periodical pressure relaxation and membrane backwash – affected human adenovirus (HAdV) removal in a bench-scale MBR. The following conclusions can be drawn:

- While both pressure relaxation and membrane backwash could alleviate fouling, the resulting increase in membrane permeability was accompanied by a decrease HAdV removal.
- Reversible fouling enhances HAdV removal in MBRs.
- During the backwash, the backwash flux (and not backwash duration) controls removal of HAdV.
- With the same ratio of filtration time to relaxation time, longer filtration/relaxation cycles lead to more fouling in a long term. However, the

differences in HAdV removal before and after pressure relaxation are not affected by the length of cycles.

- Transmembrane pressure drop (*TMP*) correlates with HAdV removal in MBRs. However, quantitative correlations between *TMP* and virus removal are dependent on the operational conditions.

References

Judd, S. (2010) The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment, Elsevier.

Hoinkis, J., Deowan, S.A., Panten, V., Figoli, A., Huang, R.R. and Drioli, E. (2012) Membrane bioreactor (MBR) technology-a promising approach for industrial water reuse. *Procedia Eng.* 33, 234-241.

Andrade, L.H., Mendes, F.D.S., Espindola, J.C. and Amaral, M.C.S. (2014) Nanofiltration as tertiary treatment for the reuse of dairy wastewater treated by membrane bioreactor. *Separ. Purif. Technol.* 126, 21-29.

Yin, Z. and Xagorarakis, I. (2015) *The Handbook of Environmental Chemistry*, Springer, Berlin Heidelberg.

Choi, J.G., Bae, T.H., Kim, J.H., Tak, T.M. and Randall, A.A. (2002) The behavior of membrane fouling initiation on the crossflow membrane bioreactor system J. *Membr. Sci.* 203(1), 103-113.

Vaid, A., Kopp, C., Johnson, W. and Fane, A.G. (1991) Integrated waste water

451 treatment by coupled bioreactor and membrane system. *Desalination* 83(1),
 452 137-143.

453 Pankhania, M., Stephenson, T. and Semmens, M.J. (1994) Hollow fibre bioreactor for
 454 wastewater treatment using bubbleless membrane aeration. *Water Res.* 28(10),
 455 2233-2236.

456 Beaubien, A., Baty, M., Jeannot, F., Francoeur, E. and Manem, J. (1996) Design and
 457 operation of anaerobic membrane bioreactors: development of a filtration
 458 testing strategy. *J. Membr. Sci.* 109(2), 173-184.

459 Kishino, H., Ishida, H., Iwabu, H. and Nakano, I. (1996) Domestic wastewater reuse
 460 using a submerged membrane bioreactor. *Desalination* 106(1), 115-119.

461 Gujer, W., Henze, M., Mino, T. and van Loosdrecht, M. (1999) Activated sludge
 462 model no. 3. . *Water Sci. Technol.* 39(1), 183-193.

463 Van der Roest, H.F., Lawrence, D.P. and Van Bentem, A.G.N. (2005) Membrane
 464 bioreactors for municipal wastewater treatment, IWA Publishing.

465 Phan, H.V., Hai, F.I., Kang, J., Dam, H.K., Zhang, R., Price, W.E., A., B. and Nghiem,
 466 L.D. (2014) Simultaneous nitrification/denitrification and trace organic
 467 contaminant (TrOC) removal by an anoxic-aerobic membrane bioreactor
 468 (MBR). *Biorresources Technol.* 165, 96-104.

469 Sun, F., Li, P. and Chen, L. (2015) Simultaneous removal of organic and nitrogen in a
 470 post-denitrification membrane bioreactor (MBR) aystem and its influential
 471 factors. *Curr. Environ. Eng.* 2, 4-10.

472 Sun, F.Y., Wang, X.M. and Li, X.Y. (2013) An innovative membrane bioreactor (MBR)

473 system for simultaneous nitrogen and phosphorus removal. *Process Biochem.*
 474 48, 1749-1756.

475 Boonnorat, J., Chiemchaisri, C., Chiemchaisri, W. and Yamamoto, K. (2014)
 476 Microbial adaptation to biodegrade toxic organic micro-pollutants in
 477 membrane bioreactor using different sludge sources. *Bioresource Technol.* 165,
 478 50-59.

479 Malaeb, L., Katuri, K.P., Logan, B.E., Maab, H., Nunes, S.P. and Saikaly, P.E. (2013)
 480 A hybrid microbial fuel cell membrane bioreactor with a conductive
 481 ultrafiltration membrane biocathode for wastewater treatment. *Environ. Sci.*
 482 *Technol.* 47, 11821-11828.

483 Bouhabila, E.H., Aïm, R.B. and Buisson, H. (2001) Fouling characterisation in
 484 membrane bioreactors. *Separ. Purif. Technol.* 22-23, 123-132.

485 Judd, S. (2008) The status of membrane bioreactor technology. *Trends Biotechnol.*
 486 26(2), 109-116.

487 Cornel, P. and Krause, S. (2008) *Advanced Membrane Technology and Applications.*
 488 Li, N.N., Fane, A.G., Ho, W.W.S. and Matsuura, T. (eds), pp. 217-238, Wiley.

489 Chang, I.S., Le Clech, P., Jefferson, B. and Judd, S. (2002) Membrane fouling in
 490 membrane bioreactors for wastewater treatment. *J. Environ. Eng.* 128(11),
 491 1018-1029.

492 Psoch, C. and Schiewer, S. (2006) Anti-fouling application of air sparging and
 493 backflushing for MBR. *J. Membr. Sci.* 283(1), 273-280.

494 Chang, I.S. and Judd, S.J. (2002) Air sparging of a submerged MBR for municipal

495 wastewater treatment. *Process Biochem.* 37(8), 915-920.

496 Yu, K., Wen, X., Bu, Q., & Xia, H. (2003). Critical flux enhancements with air
 497 sparging in axial hollow fibers cross-flow microfiltration of biologically
 498 treated wastewater. *J. Membrane Sci.*, 224(1), 69-79., Wen, X., Bu, Q. and Xia,
 499 H. (2003) Critical flux enhancements with air sparging in axial hollow fibers
 500 cross-flow microfiltration of biologically treated wastewater. *J. Membr. Sci.*
 501 224(1), 69-79.

502 Ghosh, R. (2006) Enhancement of membrane permeability by gas-sparging in
 503 submerged hollow fibre ultrafiltration of macromolecular solutions: role of
 504 module design. *J. Membr. Sci.* 274(1), 73-82.

505 Fan, F. and Zhou, H. (2007) Interrelated effects of aeration and mixed liquor fractions
 506 on membrane fouling for submerged membrane bioreactor processes in
 507 wastewater treatment. *Environ. Sci. Technol.* 41(7), 2523-2528.

508 Delgado, S., Villarroel, R. and González, E. (2008) Effect of the shear intensity on
 509 fouling in submerged membrane bioreactor for wastewater treatment. *J.*
 510 *Membr. Sci.* 311(1), 173-181.

511 Hong, S.P., Bae, T.H., Tak, T.M., Hong, S. and Randall, A. (2002) Fouling control in
 512 activated sludge submerged hollow fiber membrane bioreactors. *Desalination*
 513 143(3), 219-228.

514 Wu, J., Le-Clech, P., Stuetz, R.M., Fane, A.G. and Chen, V. (2008) Effects of
 515 relaxation and backwashing conditions on fouling in membrane bioreactor. *J.*
 516 *Membr. Sci.* 324(1), 26-32.

517 Hwang, K.J., Chan, C.S. and Tung, K.L. (2009) Effect of backwash on the
 518 performance of submerged membrane filtration. *J. Membr. Sci.* 330(1),
 519 349-356.

520 Yigit, N.O., Civelekoglu, G., Harman, I., Koseoglu, H. and Kitis, M. (2009) Effects of
 521 various backwash scenarios on membrane fouling in a membrane bioreactor.
 522 *Desalination* 237(1-3), 346-356.

523 Kim, J. and DiGiano, F.A. (2006) A two-fiber, bench-scale test of ultrafiltration (UF)
 524 for investigation of fouling rate and characteristics. *J. Membr. Sci.* 271(1),
 525 196-204.

526 Zsirai, T., Buzatu, P., Aerts, P. and Judd, S. (2012) Efficacy of relaxation, backflushing,
 527 chemical cleaning and clogging removal for an immersed hollow fibre
 528 membrane bioreactor. *Water Res.* 46(14), 4499-4507.

529 Cicek, N., Winnen, H., Suidan, M.T., Wrenn, B.E., Urbain, V. and Manem, J. (1998)
 530 Effectiveness of the membrane bioreactor in the biodegradation of high
 531 molecular weight compounds. *Water Res.* 32(5), 1553-1563.

532 Hu, J., Ong, S., Song, L., Feng, Y., Liu, W., Tan, T., Lee, L. and Ng, W. (2003)
 533 Removal of MS2 bacteriophage using membrane technologies. *Water Sci.*
 534 *Technol.* 47(12), 163-168.

535 Shang, C., Wong, H.M. and Chen, G. (2005) Bacteriophage MS-2 removal by
 536 submerged membrane bioreactor. *Water Res.* 39(17), 4211-4219.

537 Comerton, A.M., Andrews, R.C. and Bagley, D.M. (2005) Evaluation of an MBR-RO
 538 system to produce high quality reuse water: Microbial control, DBP formation

539 and nitrate. *Water Res.* 39(16), 3982-3990.

540 Fiksdal, L. and Leiknes, T. (2006) The effect of coagulation with MF/UF membrane
541 filtration for the removal of virus in drinking water. *J. Membr. Sci.* 279(1),
542 364-371.

543 Lv, W., Zheng, X., Yang, M., Zhang, Y., Liu, Y. and Liu, J. (2006) Virus removal
544 performance and mechanism of a submerged membrane bioreactor. *Process*
545 *Biochem.* 41(2), 299-304.

546 Zhang, K. and Farahbakhsh, K. (2007) Removal of native coliphages and coliform
547 bacteria from municipal wastewater by various wastewater treatment
548 processes: implications to water reuse. *Water Res.* 41(12), 2816-2824.

549 Zheng, X. and Liu, J. (2007) Virus rejection with two model human enteric viruses in
550 membrane bioreactor system. *Sci. China Ser. B: Chemistry* 50(3), 397-404.

551 Tam, L.S., Tang, T.W., Lau, G.N., Sharma, K.R. and Chen, G.H. (2007) A pilot study
552 for wastewater reclamation and reuse with MBR/RO and MF/RO systems.
553 *Desalination* 202(1), 106-113.

554 Ravindran, V., Tsai, H.H., Williams, M.D. and Pirbazari, M. (2009) Hybrid membrane
555 bioreactor technology for small water treatment utilities: process evaluation
556 and primordial considerations. *Desalination* 244(1), 39-54.

557 Hirani, Z.M., DeCarolis, J.F., Adham, S.S. and Jacangelo, J.G. (2010) Peak flux
558 performance and microbial removal by selected membrane bioreactor systems.
559 *Water Res.* 44(8), 2431-2440.

560 Chaudhry, R.M., Holloway, R.W., Cath, T.Y. and Nelson, K.L. (2015a) Impact of

561 virus surface characteristics on removal mechanisms within membrane
562 bioreactors. *Water Res.* 84, 144-152.

563 Fox, R. and Stuckey, D. (2015) MS-2 and T4 phage removal in an anaerobic
564 membrane bioreactor (AnMBR): Effect of gas sparging rate. *J. Chem. Technol.*
565 *Biotechnol.* 90, 384-390.

566 Hmaied, F., Keskes, S., Jebri, S., Amri, I., Yahya, M., Loisy-Hamon, F., Lebeau, B.
567 and Hamdi, M. (2015) Removal of rotavirus and bacteriophages by membrane
568 bioreactor technology from sewage. *Curr. Microbiol.* 71, 540-545.

569 van den Akker, B., Trinh, T., Coleman, H.M., Stuetz, R.M., Le-Clech, P. and Khan,
570 S.J. (2014) Validation of a full-scale membrane bioreactor and the impact of
571 membrane cleaning on the removal of microbial indicators. *Bioresource*
572 *Technol.* 155, 432-437.

573 Madaeni, S.S., Fane, A.G. and Grohmann, G.S. (1995) Virus removal from water and
574 wastewater using membranes. *J. Membr. Sci.* 102, 65-75.

575 Ottoson, J., Hansen, A., Björlenius, B., Norder, H. and Stenström, T.A. (2006)
576 Removal of viruses, parasitic protozoa and microbial indicators in
577 conventional and membrane processes in a wastewater pilot plant. *Water Res.*
578 40(7), 1449-1457.

579 da Silva, A.K., Le Saux, J.C., Parnaudeau, S., Pommepuy, M., Elimelech, M. and Le
580 Guyader, F.S. (2007) Evaluation of removal of noroviruses during wastewater
581 treatment, using real-time reverse transcription-PCR: different behaviors of
582 genogroups I and II. *Appl. Environ. Microb.* 73(24), 7891-7897.

583 Kuo, D.H.W., Simmons, F.J., Blair, S., Hart, E., Rose, J.B. and Xagorarakis, I. (2010)
584 Assessment of human adenovirus removal in a full-scale membrane bioreactor
585 treating municipal wastewater. *Water Res.* 44(5), 1520-1530.

586 Simmons, F.J., Kuo, D.H.W. and Xagorarakis, I. (2011) Removal of human enteric
587 viruses by a full-scale membrane bioreactor during municipal wastewater
588 processing. *Water Res.* 45(9), 2739-2750.

589 Simmons, F.J. and Xagorarakis, I. (2011) Release of infectious human enteric viruses
590 by full-scale wastewater utilities. *Water Res.* 45(12), 3590-3598.

591 Zanetti, F., De Luca, G. and Sacchetti, R. (2010) Performance of a full-scale
592 membrane bioreactor system in treating municipal wastewater for reuse
593 purposes. *Bioresource Technol.* 101(10), 3768-3771.

594 Chaudhry, R.M., Nelson, K.L. and Drewes, J.E. (2015b) Mechanisms of pathogenic
595 virus removal in a full-scale membrane bioreactor. *Environ. Sci. Technol.* 49,
596 2815-2822.

597 Wu, J., Li, H. and Huang, X. (2010) Indigenous somatic coliphage removal from a
598 real municipal wastewater by a submerged membrane bioreactor. *Water Res.*
599 44(6), 1853-1862.

600 Zheng, X., Lv, W., Yang, M. and Liu, J. (2005) Evaluation of virus removal in MBR
601 using coliphage T4. *Chinese Sci. Bull.* 50(9), 862-867.

602 Witt, D.J. and Bousquet, E.B. (1988) Comparison of enteric adenovirus infection in
603 various human cell lines. *J. Virol. Methods* 20(4), 295-308.

604 Lee, C., Lee, S.H., Han, E. and Kim, S.J. (2004) Use of cell culture-PCR assay based

605 on combination of A549 and BGMK cell lines and molecular identification as
 606 a tool to monitor infectious adenoviruses and enteroviruses in river water.
 607 *Appl. Environ. Microb.* 70(11), 6695-6705.

608 Yin, Z., Tarabara, V.V. and Xagorarakis, I. (2015) Human adenovirus removal by
 609 hollow fiber membranes: Effect of membrane fouling by suspended and
 610 dissolved matter. *J. Membr. Sci.* 482, 120-127.

611 Yuan, Q., Sparling, R. and Oleszkiewicz, J.A. (2009) Waste activated sludge
 612 fermentation: effect of solids retention time and biomass concentration. *Water*
 613 *Res.* 43(20), 5180-5186.

614 Broughton, A., Pratt, S. and Shilton, A. (2008) Enhanced biological phosphorus
 615 removal for high-strength wastewater with a low rbCOD: P ratio. *Bioresource*
 616 *Technol.* 99(5), 1236-1241.

617 Heim, A., Ebnet, C., Harste, G. and Pring-Åkerblom, P. (2003) Rapid and quantitative
 618 detection of human adenovirus DNA by real-time PCR. *J. Med. Virol.* 70(2),
 619 228-239.

620 Sutlović, D., Definis-Gojanović, M., Anđelinović, Š., Gugić, D. and Primorac, D.
 621 (2005) Taq polymerase reverses inhibition of quantitative real time polymerase
 622 chain reaction by humic acid. *Croat. Med. J.* 46(4), 556-562.

623 Wen, C., Huang, X. and Qian, Y. (1999) Domestic wastewater treatment using an
 624 anaerobic bioreactor coupled with membrane filtration. *Process Biochem.*
 625 35(3), 335-340.

626 Xagorarakis, I., Yin, Z. and Svambayev, Z. (2014) Fate of viruses in water systems. *J.*

627 Environ. Eng. ASCE 140(7), 04014020.

628

Clean membrane

Fouled membrane

After pressure relaxation
(reduced foulant on membrane surface)

After permeate backwash
(reduced foulant in membrane pores)