



Impact of virus surface characteristics on removal mechanisms within membrane bioreactors



Rabia M. Chaudhry^{a, b}, Ryan W. Holloway^{a, c}, Tzahi Y. Cath^{a, c}, Kara L. Nelson^{a, b, *}

^a ReNUWIt (Reinventing the Nation's Urban Water Infrastructure), Engineering Research Center, University of California, Berkeley, CA 94720, USA

^b Department of Civil and Environmental Engineering, University of California, Berkeley, CA 94720, USA

^c Department of Civil & Environmental Engineering, Colorado School of Mines, Golden, CO 80401, USA

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ABSTRACT

In this study we investigated the removal of viruses with similar size and shape but with different external surface capsid proteins by a bench-scale membrane bioreactor (MBR). The goal was to determine which virus removal mechanisms (retention by clean backwashed membrane, retention by cake layer, attachment to biomass, and inactivation) were most impacted by differences in the virus surface properties. Seven bench-scale MBR experiments were performed using mixed liquor wastewater sludge that was seeded with three lab-cultured bacteriophages with icosahedral capsids of ~30 nm diameter (MS2, phiX174, and fr). The operating conditions were designed to simulate those at a reference, full-scale MBR facility. The virus removal mechanism most affected by virus type was attachment to biomass (removals of 0.2 log for MS2, 1.2 log for phiX174, and 3 log for fr). These differences in removal could not be explained by electrostatic interactions, as the three viruses had similar net negative charge when suspended in MBR permeate. Removals by the clean backwashed membrane (less than 1 log) and cake layer (~0.6 log) were similar for the three viruses. A comparison between the clean membrane removals seen at the bench-scale using a virgin membrane (~1 log), and the full-scale using 10-year old membranes (~2–3 logs) suggests that irreversible fouling, accumulated on the membrane over years of operation that cannot be removed by cleaning, also contributes towards virus removal. This study enhances the current mechanistic understanding of virus removal in MBRs and will contribute to more reliable treatment for water reuse applications.

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1. Introduction

A mechanistic understanding of virus removal in membrane bioreactors (MBRs) is essential to determine their effectiveness as components of treatment trains for potable and non-potable water reuse. Pathogens pose an acute public health threat, and minimizing that risk is crucial for further expanding water reuse as an acceptable approach for sustainably managing strained urban water resources. MBRs are becoming popular as a wastewater technology for water reuse because they combine biological treatment and membrane separation (e.g., microfiltration (MF) or ultrafiltration (UF)) into one unit process, and provide better and more

consistent effluent quality than conventional activated sludge (Radjenović et al., 2008; Wachinski, 2013). MBRs are expected to have greater removal of larger pathogens, such as protozoa and bacteria, than viruses, because they are larger than the nominal membrane pore sizes (Ottozon et al., 2006), whereas viruses may be capable of passing through membrane pores due to their smaller size. Virus removal by MBRs is thought to occur via four mechanisms: (i) incorporation of viruses into the mixed liquor suspended solids, which are excluded by the membrane, (ii) retention of viruses by the clean backwashed membrane, (iii) retention of viruses by the cake layer formed on the membrane surface after a period of operation, and (iv) inactivation of the viruses within mixed liquor due to extracellular enzymes and predation (Chaudhry et al., 2015). However, it is unclear how viruses with different morphologies and surface characteristics are affected by each of these mechanisms, and whether any differences in treatment through the MBR process for the myriad of viruses present in wastewater is a cause for concern.

* Corresponding author. ReNUWIt (Reinventing the Nation's Urban Water Infrastructure), Engineering Research Center, University of California, Berkeley, CA 94720, USA.

E-mail address: karanelson@berkeley.edu (K.L. Nelson).

Several studies have demonstrated the ability of full-scale MBRs to achieve over 4-log removal of virus surrogates and bacteriophages (De Luca et al., 2013; Francy et al., 2012; Van den Akker et al., 2014; Zanetti et al., 2010) as well as pathogenic viruses (Chaudhry et al., 2015; Da Silva et al., 2007; Kuo et al., 2010; Sima et al., 2011; Simmons et al., 2011) under long-term steady state conditions. Two pilot studies have demonstrated improvement in seeded phage removal after a period of operation (Trussell et al., 2012) and as trans-membrane pressure increased (TMP) (Marti et al., 2011), indicating the buildup of a protective cake layer on the membrane. However, virus removal mechanisms in MBRs have been investigated only by few studies (Chaudhry et al., 2015; Lu et al., 2013; Lv et al., 2006; Shang et al., 2005; Ueda and Horan, 2000; Wu et al., 2010; Zheng and Liu, 2006). The relevant results from studies that reported mechanistic log removal values are summarized in Table 1. Furthermore, due to differences in the scale of experiments, types of target viruses, and operating conditions, it is difficult to determine which removal mechanism is most susceptible to differences in virus morphology and surface characteristics.

Virus interactions with environmental surfaces such as fecal material, clays, and biological flocs are important in determining their attachment behavior, and consequently their removal during treatment. The outer layer of enteric viruses that are typically found in wastewater consists of protein capsids composed of polypeptides that include weakly acidic and basic amino acids, and the pH-dependent dissociation of these functional groups imparts a net charge to the capsids. The pH at which the capsid is uncharged is called the isoelectric point (IEP) (Voyles, 2002). Classic DLVO theory for colloid stability has been invoked to model virus behavior and transport. Electrostatic interactions between two charged particles in dispersion are thought to result from a balance between the repulsive electrostatic double-layer interactions and the attractive van der Waals forces (Shaw, 2000). The model assumes a uniformly charged, flat surface, but discrete charges such as ions or a more complex virus particle with a heterogeneous surface may behave differently.

Recent work investigating pathogenic viruses and phages under controlled solution chemistries (divalent cations, ionic strength, natural organic matter, etc.) has demonstrated the importance of individual virus surface properties in determining attachment and aggregation behavior (Armanious, 2014; Gutierrez et al., 2010, 2009; Mylon et al., 2010; Pham et al., 2009). Controlled laboratory studies with viruses also suggest that hydrophobic effects (Gerba, 1984; Templeton et al., 2008) and steric impacts of structural components (Redman et al., 1997) can also play a significant role in virus-surface interactions. Therefore, viruses with unique surface properties are hypothesized to have different attachment behaviors under the same conditions. However, it is challenging to extrapolate the results of laboratory studies with well-controlled conditions to the complex environment of a real wastewater treatment system.

The objectives of this study were to determine: (i) how viruses with the same size and shape but with different surface characteristics are removed in a bench-scale MBR process using real wastewater; and (ii) which virus removal mechanisms (retention by membrane, retention by cake layer, attachment to biomass, and inactivation) are affected by differences in virus surface characteristics. The experimental design and operational parameters were based on a full-scale study, which serves as a reference (Chaudhry et al., 2015). We used three phages (MS2, phiX174, and fr), which are all approximately 30 nm in diameter with naked icosahedral protein capsids. MS2 and fr are members of the F+ coliphages quantified in the reference study, and have single-

Table 1 Summary of mechanism contributions towards log removal in an MBR process from this and previous studies. The contribution of indigenous decay for this study is included in the biomass LRV. PE = polyethylene, PVDF = polyvinylidene fluoride. The conditions in this study were modeled as closely to the full-scale study (Chaudhry et al., 2015) as possible to facilitate comparison.

Parameter	Ueda and Horan, 2000	Shang et al., 2005	Wu et al., 2010	Chaudhry et al., 2015	Chaudhry et al., 2015	Chaudhry et al., 2015	This Study	This Study	This Study
Membrane supplier and type	Kubota, flat sheet	Mitsubishi, hollow fiber	Mitsubishi, hollow fiber	ZeeWeed hollow fiber	ZeeWeed hollow fiber	ZeeWeed hollow fiber	KMS hollow fiber	KMS hollow fiber	KMS hollow fiber
Study parameters	Bench scale	Bench scale, synthetic wastewater	Bench scale	Full-scale	Full-scale	Full-scale	Bench scale	Bench scale	Bench scale
Nominal membrane pore (µm) and type	0.4 (PE)	0.4 (PE)	0.4 (PE)	0.04 (PVDF)	0.04 (PVDF)	0.04 (PVDF)	0.04 (PVDF)	0.04 (PVDF)	0.04 (PVDF)
Virus	Indigenous somatic coliphage	Lab-grown MS2	Indigenous somatic coliphage	Indigenous adenovirus	Indigenous norovirus GII	Indigenous F+ coliphage	Lab-grown MS2	Lab-grown phiX174	Lab-grown fr
Phage size (nm)	200	24	80	30	30	Most likely 30	30	30	30
Membrane LRV contribution	0.1–0.3	0.4	2.0	3.1	2.7	2.7	0.9	0.4	0.5
Cake layer LRV contribution	3.58	2.1	0.4–1.6	0.2–0.5	0.8–1.6	0.8–1.6	0.6	0.7	0.7
Biomass LRV contribution	2.2	0.8	0.8	0.5	1.0	1.0	0.2	1.2	3.0
First-order decay constant k (hr ⁻¹)	–	–	0.4	1.3	2.0	2.0	0.22	0.13	0.07

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