Journal of Membrane Science 497 (2016) 120–127

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Effect of virus influent concentration on its removal by microfiltration: The case of human adenovirus 2

Ruiqing Lu [a], Qi Li [a,c], Ziqiang Yin [b], Irene Xagoraraki [b], Volodymyr V. Tarabara [b], Thanh H. Nguyen [a,*]

[a] Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, United States
[b] Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI 48824, United States
[c] School of Urban and Environmental Science, Northwest University, Xi'an 710127, China

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form
28 August 2015
Accepted 30 August 2015
Available online 3 September 2015

Keywords:
Human adenovirus
Microfiltration
Virus removal
Membrane fouling

ABSTRACT

For safe water reuse, pathogenic viruses need to be efficiently controlled. Membrane filtration is considered to be an effective technology for virus removal. The present work explores mechanisms of human adenovirus 2 (HAdV-2) removal by a hollow fiber microfiltration membrane (d_{pore} = 0.2 μm) as a function of influent virus concentration ranging from 1.3×10^7 to 3.4×10^8 copies/mL. A gradual decrease of HAdV-2 removal over time was observed at the beginning of filtration and was attributed to the accumulation of rejected HAdV-2 at the membrane surface or within membrane pores. Flux decline analysis revealed that complete pore blocking and standard pore blocking dominated the early stages of filtration and then transited to cake filtration at longer filtration time as the primary reason for flux decline. Deposition of HAdV-2 onto the membrane during the later stages of filtration led to the formation of a partly irreversible fouling layer and an increase of HAdV-2 removal. The understanding of HAdV-2 behavior at the membrane surface revealed in this study can help with the development of antifouling membranes with high virus removal efficiency.

1. Introduction

With population and water demand growing, ever increasing water scarcity has driven water reuse in the United States and other countries [1]. Municipal wastewater is currently regarded as an important resource rather than waste. Treatment technologies have enabled non-potable reuse of treated municipal wastewater for irrigation, aquifer recharge, and application in various industries [2]. The potable reuse, however, is still very limited in practice [3]. For both potable and non-potable reuse of municipal wastewater, a sufficient removal of pathogens, including the smallest microorganisms – viruses-is required to ensure public safety. For example, human adenovirus (HAdV), one of the most common waterborne viral pathogens [4–6], was found at concentrations higher than 10^3 virus/L by qPCR even after disinfection at five wastewater treatment plants in Michigan [7]. These results suggest the need for novel advanced technology instead of conventional treatment to ensure virus removal in wastewater treatment facilities. Full scale membrane bioreactors (MBRs) were proven to achieve better virus removal compared to conventional wastewater treatment, which relies on the activated sludge process as the secondary treatment [7]. However, the major challenge with membrane filtration, especially for wastewater treatment, is the cost associated with controlling membrane fouling [8–10]. For this reason, significant efforts have been put forth to understand fouling mechanisms [11–13] and to develop fouling-resistant membranes and operational strategies that minimize permeate flux decline caused by membrane fouling [14–19].

The efficiency of virus removal by membranes of different pore sizes has been examined using bacteriophages as surrogates for pathogenic viruses. Microfiltration membranes achieve 1–2 logs (90–99%) virus removal while affording much higher permeate flux than ultra- or nanofiltration membranes [20,21]. New microfiltration membrane designs have been explored to achieve high virus removal without losing the benefit of the high permeate flux, and efforts have been made to incorporate virus removal as a criteria in developing new membranes [22]. However, the lack of detailed understanding of virus removal mechanisms prevents these efforts from translating into practical benefits. Membrane separation and fouling are essentially the consequences of surface interactions. Therefore, knowledge on virus behavior in the vicinity of the membrane surface is important for achieving an optimal combination of virus removal and high permeate flux. HAdV-2 are biological colloids that consist of a protein capsid and a double

* Corresponding author.
E-mail address: thn@illinois.edu (T.H. Nguyen).

http://dx.doi.org/10.1016/j.memsci.2015.08.065
0376-7388/

stranded DNA in the virus core [23]. Previous findings on either model colloids [24,25] or model proteins [26,27] can be helpful but insufficient for understanding the behavior of HAdV-2 in the membrane surface vicinity.

To fill this knowledge gap, we studied the effects of virus concentration near the membrane surface on virus removal. A bench-scale microfiltration unit was employed to investigate the dynamics of virus removal and membrane permeate flux as functions of influent concentration of the virus. We used human adenovirus type 2 (HAdV-2) as a model pathogenic virus, because HAdV-2 was frequently detected in municipal wastewater [4,28,29]. We hypothesize that during the initial stage of filtration, HAdV-2 removal efficiency is influenced by the influent concentration of the virus, as found for other model colloids [30]. This hypothesis was tested by monitoring permeate flux and HAdV-2 removal over the filtration time for different influent concentrations of HAdV-2. If the flux decline is hydraulically reversible (i.e., the permeate flux can be recovered to that of the clean membrane when the transmembrane pressure differential decreased to zero), we expect that HAdV-2 removal would also be recovered [24]. If the flux decline is hydraulically irreversible, and a gel or cake layer is formed on the membrane surface [25], we expect that HAdV removal would be affected by the fouling layer. We applied a combined pore blockage–cake filtration model (PB–CF) [26] to elucidate virus behavior in the immediate vicinity of membrane surface. By correlating the observed virus removal and fouling mechanisms revealed by the PB–CF model, we draw conclusions on how and why virus removal depends on the influent virus concentration and the filtration time. Future efforts will focus on HAdV removal in the presence of other foulants and by membranes with antifouling surfaces.

2. Methods

2.1. HAdV-2 propagation and purification

HAdV-2 (ATCC, VR-846) was propagated in the A549 human lung carcinoma cells (Diagnostic Hybrids). Before inoculation with HAdV-2, the A549 cells were propagated in cell culture flasks at 37 °C in a 5% CO_2 incubator. The growth medium used for A549 cell propagation was Ham's F12K growth media supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B [31]. Once the A549 cells in the flask reached 80–90% confluence, they were inoculated with HAdV-2. The growth media used for HAdV-2 amplification was similar to the one used for A549 propagation except that 2% FBS was supplemented. The cytopathic effect of the A549 cells was observed 3 to 4 days after inoculation. HAdV-2 was released from the A549 cells by 3 cycles of freezing in −80 °C and thawing in 4 °C. The suspension after lysis was centrifuged at 230*g* for 10 min to precipitate cell debris. The supernatant was filtered with a 0.45 μm pore size cellulose acetate membrane (Corning CLS431155) to further remove cell debris. The filtrate was concentrated and further purified using a 100 kDa ultrafiltration membrane (Koch HFM-180) housed in an Amicon stirred cell (Millipore) to remove residues of the growth media. By repeatedly adding sterile 1 mM $NaHCO_3$ solution into the stirred cell, the media residue in the HAdV-2 suspension was washed away. The concentration of HAdV-2 in the resulting stock suspension was measured with quantitative real-time PCR (qPCR) as described below.

2.2. HAdV-2 hydrodynamic diameter measurements

The measurements of HAdV-2 hydrodynamic diameter were performed using a method similar to a procedure described previously [32,33]. Briefly, dynamic light scattering (DLS) measurements were conducted using Zetasizer (ZS90, Malvern). The concentration of HAdV-2 in the measurement was diluted to 3×10^8 virus/mL in 3 mM $CaCl_2$ solution at pH=8.0 buffered by 1 mM $NaHCO_3$. The ionic composition and pH were chosen to match those in our previous measurement of municipal wastewater from a full-scale wastewater treatment plant in Traverse City, Michigan [32]. The measurements were performed immediately after the sample preparation and 1 day afterwards to study whether HAdV-2 had aggregated. Each measurement included three replicates.

2.3. Quantification of HAdV-2 by qPCR

qPCR was applied to quantify HAdV-2 concentration with a protocol modified from the one reported previously [34]. The genome of HAdV-2 was extracted with PureLink Viral RNA/DNA Mini Kit (Invitrogen, USA). The conserved Hexon gene in the HAdV-2 genome (nt. 18856–19137 in HAdV-2 sequence) was the qPCR amplification region. Primers for the amplification region are as follows: AQ1 (5′-GCC-ACG-GTG-GGG-TTT-CTA-AAC-TT-3′) and AQ2 (5′-GCC-CCA-GTG-GTC-TTA-CAT-GCA-CAT-C-3′). Templates of qPCR standard curves were acquired with the double stranded DNA of the Hexon gene. This DNA standard was produced by Integrated DNA Technologies (Coralville, IA). A blank sample with molecule biology grade water (Corning) was always included with standard curves and unknown samples. The reaction mix of qPCR included 7.5 μL Power SYBR Green PCR Master Mix (Life Technology, NY), 2 μL templates (standards, samples or blank), 1.5 μL of each primer to get a final concentration of 0.5 μM, and 2.5 μL of PCR-grade water. Reaction was performed on a BioRad MiniOptical Real-Time PCR system. The reaction started with 95 °C for 10 min to activate DNA polymerase, followed by 45 cycles of denaturation at 95 °C for 3 s, annealing at 55 °C for 10 s and extension at 60 °C for 1 min with fluorescence signal measured at the end of each cycle. The threshold of fluorescence signal and cycle numbers to achieve the threshold for each sample were calculated automatically by Sequence Detection Systems Software 2.4 (Applied Biosystems, CA). For standard curves, qPCR reactions were run with serial dilutions of the Hexon gene DNA standard as templates at concentration from 1.3×10^2 to 1.3×10^6 genome equivalent (copies)/run. The reaction of 1.3×10^1 copies/run was only occasionally detected, while concentrations higher than or equal to 1.3×10^2 copies/run were constantly detected. Blank samples were not detected in all reactions. A standard curve was generated with freshly prepared serial dilution of the Hexon gene DNA standard for every set of qPCR. Using the cycle numbers achieving the threshold as the *y* axis and the $\log_{10}$ value of gene copy numbers as the *x* axis, the average slope was -3.43 ± 0.12 for 10 standard curves in total, and the coefficients of determination (R^2) were all higher than 0.99. The average amplification efficiency was 95% calculated from the average slope of the standard curves.

2.4. HAdV-2 removal by a hollow fiber microfiltration membrane

The HAdV-2 removal and the permeate flux were monitored in filtration experiments that used a hollow fiber filtration unit operated in the constant pressure mode (Fig. 1), as described in previous studies [32,35]. The pressure controller installed in the retentate line downstream of the membrane kept the transmembrane pressure at 0.4 bar throughout the filtration process. Filtration experiments were conducted in the constant pressure mode instead of constant flux mode to check whether the PB–CF model can be applied to the flux data to determine HAdV-2 fouling mechanisms [26]. The influent was pumped into the filtration unit by