HUMAN ADENOVIRUS REMOVAL IN WASTEWATER TREATMENT AND MEMBRANE PROCESS

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ABSTRACT

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Occurrence of human pathogenic viruses in environmental waters (i.e., surface waters, groundwater, drinking water, recreational water, and wastewater) raises concerns regarding the possibility of human exposure and waterborne infections. Presence of virus in water and wastewater is a difficult problem for environmental engineers because of prevalence, infectivity, and resistance of viruses to disinfection. On the other hand, it has been suggested that development of membrane Technology in treating municipal wastewater, such as membrane bioreactors, provides high quality effluents appropriate for water reuse.

Removal of human adenovirus 40 (HAdV 40) by hollow fiber ultrafiltration (UF, $d_{pore} = 0.04 \ \mu m$) and microfiltration (MF1, $d_{pore} = 0.22 \ \mu m$; MF2, $d_{pore} = 0.45 \ \mu m$) membranes was evaluated in humic acid (model dissolved species), SiO$_2$ microspheres (model suspended species) and a mix of these constituents. Three separate effects are identified: 1) increased removal due to pore blockage by dissolved species; 2) decreased removal due to cake-enhanced accumulation of viruses near membrane surface; and 3) increased removal by the composite cake acting as a secondary membrane. Comparing to the extent of fouling, feed water composition and membrane pore size together plays more important role in virus removal. Pore blockage improves virus removal while cake formation can either increase or decrease virus removal depending on the relative permeability of the cake.

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 ($t_{\Delta P>0}$), pressure relaxation ($t_{\Delta P=0}$) and backwash ($t_{\Delta P<0}$) steps. Both pressure relaxation and permeate backwash led to significant reductions in virus removal. For the same value of $t_{\Delta P>0}/t_{\Delta P=0}$, longer filtration/relaxation cycles (i.e. larger $t_{\Delta P} + t_{\Delta P=0}$) led to higher transmembrane pressure ($\Delta P$) but did not have a significant impact on virus removal. A shorter backwash ($t_{\Delta P<0} = 10$ min) at a higher flow rate ($Q = 40$ mL/min) resulted in more substantial decreases in $\Delta P$ and virus removal than a longer backwash ($t_{\Delta P<0} = 20$ min) at a lower flow rate ($Q = 20$ mL/min) even though the backwash volume ($Q t_{\Delta P<0}$) was the same. Virus 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 $\Delta P$ and virus removal.

Virus adsorption to sludge particles has been suggested as one of the major mechanisms of virus removal. Our results showed that adsorption of HAdV to primary and secondary sludge conformed to Freundlich isotherm, and it exhibited very similar behavior in the two types of sludge. More HAdV was desorbed from primary sludge during sequential desorption experiments, but the difference was not statistically significant. Greater HAdV adsorption was observed when sludge filtrate was used as solute compared to DI water.
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**KEY TO ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BOD</td>
<td>Biochemical Oxygen Demand</td>
</tr>
<tr>
<td>CAS</td>
<td>Conventional Activated Sludge</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>EBPR</td>
<td>Enhanced Biological Phosphorus Removal</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular Polymeric Substances</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>GE</td>
<td>General Electric</td>
</tr>
<tr>
<td>IWMI</td>
<td>International Water Management Institute</td>
</tr>
<tr>
<td>HA</td>
<td>Humic Acid</td>
</tr>
<tr>
<td>HAdV</td>
<td>Human Adenovirus</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>MBR</td>
<td>Membrane Bioreactor</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed Liquor Suspended Solids</td>
</tr>
<tr>
<td>NRC</td>
<td>National Research Council</td>
</tr>
<tr>
<td>NTU</td>
<td>Nephelometric Turbidity Unit</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>SMP</td>
<td>Soluble Microbial Products</td>
</tr>
<tr>
<td>SNdN</td>
<td>Simultaneous Nitrification and Denitrification</td>
</tr>
<tr>
<td>SRT</td>
<td>Solids Retention Time</td>
</tr>
<tr>
<td>TMP</td>
<td>Trans-membrane Pressure</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>TKN</td>
<td>Total Kjeldahl Nitrogen</td>
</tr>
<tr>
<td>TN</td>
<td>Total Nitrogen</td>
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TP - Total Phosphate
TSS - Total Suspended Solids
USEPA - United States Environmental Protection Agency
UV - Ultraviolet
WHO - World Health Organization
WWTP - Wastewater Treatment Plant
CHAPTER 1

BACKGROUND

1.1. Viruses of Concern in the U.S.

1.1.1. Waterborne viruses and potential human diseases

Viruses are the most abundant microorganisms on the earth (Madigan and Martinko 2006). It has been suggested that more than 150 types of enteric viruses are excreted in human feces and may be present in contaminated waters (Wong et al. 2012a; Leclerc et al. 2000; Havelaar et al. 1993). Enteric viruses are usually transmitted to humans by oral ingestion (Tanni et al. 1992). Infection by viruses may lead to various diseases, including gastroenteritis, heart anomalies, meningitis, conjunctivitis, hepatitis, and respiratory diseases (Crites and Tchobanoglous 1998; Swenson et al. 2003). Waterborne viral infections can be fatal to sensitive populations such as children, the elderly, and the immune-compromised. Waterborne disease statistics reflect a growing global burden of infectious diseases from contaminated drinking water, while ingestion of surface water during recreational activities is also a common exposure pathway to viruses and other pathogens. Viruses are contaminants of concern that may be regulated in the future, as indicated by their presence on Environmental Protection Agency (EPA)’s contaminant candidate lists (Table 1-1).

Table 1-1 also includes the classification for these waterborne viruses. Generally, there are two major systems for virus classification. One system is authorized and organized by the International Committee on Taxonomy of Viruses (ICTV). Based on both genome type and sequence similarity, ICTV classification divides viruses in orders (-virales), families (-viridae), subfamilies (-virinae), genera (-virus), and species (Korsman et al. 2012). The current (2012) ICTV taxonomy includes 7 orders, 96 families, 22 subfamilies, 420 genera, and 2618 species (ICTV, 2012). Another system is called Baltimore classification, which
classifies viruses into seven groups with different types of hosts (animal, plant, bacteria, algae, fungi and protozoa) on the basis of genome type and replication strategy. Most virus families are included in Groups I – V, whereas only a few families belong to Groups VI and VII (Dimmock et al. 2001).

Human adenoviruses are important opportunistic pathogens in immunocompromised patients (Wadell 1984) and have been identified as etiological agents in several waterborne outbreaks (Foy et al. 1968; D’Angelo et al. 1979; Martone et al. 1980; Kukkula et al. 1997; Papapetropoulou and Vantarakis 1998; Borchardt et al. 2003a). Diseases caused by human adenoviruses include conjunctivitis, ocular infections, gastroenteritis, respiratory disease, encephalitis, pneumonia, genitourinary infections, and pharyngoconjunctival fever. The potential health risk to infants, children and adults, associated with adenovirus waterborne transmission are confirmed by the scientific community (Irving and Smith 1981; Albert 1986; Uhnoo et al. 1986; Adrian et al. 1987; Hurst et al. 1988; Krajden et al. 1990; Cruz et al. 1990; Enriquez et al. 1995; Horwitz 1996; Foy 1997; Bon et al. 1999; Borchardt et al. 2003a; Swenson et al. 2003).

It has been reported that enteroviruses are responsible for most outbreaks of enteroviral meningitis (Abzug et al. 2003; Rotbart 2000). Poliovirus is a type of human enterovirus mainly causing poliomyelitis (Madaeni et al. 1995). Coxsackievirus usually causes “hand-foot-and-mouth disease” in young children, and it can be fatal for people with weak immune systems. Echovirus is a subspecies of enterovirus B, and it is a usual cause of aseptic meningitis (Martinez et al. 2012; Xiao et al. 2013). Symptoms of infection by hepatitis A virus vary greatly, and severe cases of infection can cause death. Person-to-person contact is an important transmission path in addition to fecally contaminated food and water (Morace et al. 2002; Cuthbert 2001). Hepatitis virus has a prolonged incubation period in cell cultures and polymerase chain reaction (PCR) is suggested as a preferable method for HAV detection (Divizia et al. 1998).
Caliciviruses cause various diseases in animals, including gastroenteritis, respiratory infections, vesicular lesions, hemorrhagic disease, while the associated disease in humans is mainly gastroenteritis (Farkas et al. 2008). Noroviruses are the most common etiologic agents in *caliciviridae* family. They are highly contagious, and the required dose for viral infection is very low (Ausar et al. 2006). One challenge in norovirus studies is that high concentrations of noroviruses cannot be easily produced since they are not culturable (Farkas et al. 2008).

Rotavirus has been recognized as one of the most common causes of acute infectious gastroenteritis (Marshall 2009) and the leading cause of severe, dehydrating diarrhea in children (WHO 2007). Outbreaks of viral gastroenteritis caused by rotaviruses have been reported in both infants and adults (Craun et al. 2010; Anderson and Weber 2004; Siqueira et al. 2010), and rotaviruses might be responsible for more than 50% of enteritis among infants worldwide (Fenner and White 1976).

### 1.1.2. Waterborne outbreaks related to viruses

It has been reported that 1.5-12 million people die per year from waterborne diseases (Gleick 2002; WHO 2004). Most of the waterborne outbreaks in the US have been related to microbial agents (Moore et al. 1993; Kramer et al. 1996; Levy et al. 1998; Barwick et al. 2000; Lee et al. 2002; Yoder et al. 2004; Liang et al. 2006), and over the last decade, thousands of people in the United States have experienced waterborne diseases. The majority of the outbreaks involved unidentified agents. The Environmental Protection Agency suspects that many of the outbreaks due to unidentified sources were caused by enteric viruses (USEPA 2006). Ground water is an important transmission route for waterborne viral infections (USEPA 2006). The majority of outbreaks associated with drinking water are caused by water from wells, while outbreaks associated with recreational water mainly occur in natural water bodies. Since 1980, over 70 outbreaks of diseases in the United States
reported by the CDC have been attributed to viruses, and it is estimated that the actual number of outbreaks is a lot higher. It is believed that the role of viruses associated with waterborne disease is underestimated since their occurrences are under-reported and it is difficult to specify the agents (Mena et al. 2007).

Noroviruses (Norwalk-like virus) appear to be the most common aetiological agents of gastroenteritis in the United States and are responsible for more than half of both recreational and drinking water outbreaks (Blackburn et al. 2004; Yoder et al. 2008a; Brunkard et al. 2011; Barwick et al. 2000; Lee et al. 2002; Yoder et al. 2004; Dziuban et al. 2006; Yoder et al. 2008b; Hlavsa et al. 2011). Outbreaks caused by Hepatitis A viruses are also frequently reported by CDC and are mostly associated with drinking water as opposed to recreational water exposure (Kramer et al. 1996; Moore et al. 1993; Yoder et al. 2008a; Brunkard et al. 2011; Mahoney et al. 1992). Three outbreaks reported by CDC were caused by adenoviruses. One was in 1982 and two were in 1991. All were related to recreational water, and the associated diseases include conjunctivitis and Pharyngitis (Turner et al. 1987; Moore et al. 1993). Enteroviruses (coxsackievirus, echovirus) were reported as aetiological agents in three outbreaks (Hejkal et al. 1982; Levine et al. 1990; Dziuban et al. 2006), two of which were related to recreational water. Associated diseases include meningitis and gastroenteritis. Rotaviruses were the cause of one outbreak in Colorado, and tap water was identified as the contamination source (Hopkins et al. 1985). Outbreaks of hepatitis E were reported in other countries (Corwin et al. 1996), but the United States is considered a non-endemic area for hepatitis E (Favorov et al. 1992; Favorov et al. 1999; Aggarwal and Krawczynski 2000), and outbreaks due to hepatitis E haven’t been reported (Hughes et al. 2010). However, sporadic cases of hepatitis E infection have been observed (Tsang et al. 2000; Kwo et al. 1997; Munoz et al. 1992), and some of the patients had no history of travelling outside the U.S. (Tsang et al. 2000). Swine are known as a reservoir of hepatitis E, and also a potential source for virus transmission to human (Colson et al. 2010; Dong et al. 2011).
1.2. Source and Fates of Viruses in the Environment

1.2.1. Sources of viruses in the environment

The sources and reservoirs of human viruses are shown in Figure 1-1. Human enteric viruses are frequently found in surface water, and the sources of viruses could be effluent from wastewater treatment plants, combined sewer overflows, leaching septic systems, and runoff from agriculture areas. Runoff and infiltration during precipitation events can lead to viral contamination of surface and groundwater. In the case of permeable soils, the most likely route of pollutant transfer is through the soil to groundwater. Preferential flow paths caused by plant roots, cracks, fissures and other natural phenomena can rapidly move viral contaminants to shallow groundwater.

Wastewater is one of the most concentrated sources of infectious viruses (Puig et al. 1994, Castignolles et al. 1998). The estimated mean concentration of enteric viruses in wastewater in the United States is approximately 7000 infectious viruses per liter (Melnick et al. 1978), and the highest concentrations of viral particles can reach $10^9$ per liter (da Silva et al. 2007; Kuo et al. 2010; Simmons et al. 2011). Wastewater utilities may release viruses to environmental waters via treated effluent discharge and biosolids that are land applied. During rainfall events, untreated sewage and wastewater may be directly discharged into surface water in combined sewer overflows (Donovan et al. 2007).

Fecal contamination from livestock manure handling and storage facilities is one of the most important sources of groundwater microbiological pollution (USEPA 2006). Manure and other animal wastes contain high concentrations of infectious zoonotic viruses, protozoa, and bacteria (Meslin 1997; Slifko et al. 2000; Sobsey et al. 2001; Hubalek 2003; Gannon et al. 2004; Cliver and Moe 2004; Palmer et al. 2005). Zoonotic viruses from animals may cause diseases in humans. For example, hepatitis E is considered as a zoonotic virus, of which the potential transmission from animal, such as swine, to human has been proposed (Clayson et al. 1996; Wu et al. 2000).
1.2.2. Viruses as microbial source tracking tools

Traditional microbial indicators are widespread in the environment, and the related measurements are simple. However, the most significant deficiency of *E. coli* and *enterococci* as MST tools is lack of host specificity (Ahmed et al. 2007; Gordon et al. 2001). Microbial source tracking (MST) is a relatively new, fast developing technology that allows people to discriminate among possible sources of fecal contamination in the environment (Hagedorn et al. 2011). A number of microorganisms have been proposed as candidate tools for MST.

Human adenovirus (HAdV), human enterovirus (HEV), and human polyomavirus (HPyV), have been suggested as potential MST tools indicating human pollution sources (Harwood et al. 2009; Noble et al. 2003; Ahmed et al. 2010). Fong et al. (2005) characterized HAdVs and HEVs as sound library-independent indicators that can be used for the identification of water pollution sources. After analyzing pig slaughterhouse slurries, urban sewage and river water samples, Hundesa et al. (2006 and 2009) suggested that porcine adenoviruses (PAdVs) detection provides a valuable MST approach. Also, HPyVs are highly human specific, so that their detection provides a reliable indication of contamination from a human source (Harwood et al. 2009). Bovine adenovirus (BAdV) and bovine enterovirus (BEV) were proposed for use in identifying agricultural water pollution sources (Ahmed et al. 2010; Fong et al. 2005). Bovine polyomavirus (BPyV) has been characterized as a particularly robust MST tool (Hundesa et al. 2010) that might perform better than BAdV at sites where manure is a suspected source of contamination (Wong and Xagoraraki 2011). Moreover, some types of bacteriophages, such as F RNA specific phage (Lee et al. 2009; Smith et al. 2006; Stewart et al. 2006; Gourmelon et al. 2010), were also suggested as potential MST tools. The occurrence and concentration of human and animal viruses are fairly low in fresh water bodies. In order to make viruses detectable and efficiently use them as MST tools, a concentration procedure is usually required involving filtration of large amounts of water during sampling.
1.2.3. Viruses in natural water bodies, sediments, and soils

Numerous studies have found human enteric viruses in surface water in many countries including well developed, industrialized countries (De Paula et al. 2007; Xagoraraki et al. 2007; Jiang et al. 2007; Miagostovich et al. 2008; Chen et al. 2008; Shieh et al. 2008; Costan-Longares et al. 2008). As an example, occurrences of enteric viruses have been reported in fresh water in the Great Lakes region. Human adenoviruses were the most frequently detected viruses at Great Lakes beaches (Fong et al. 2007; Aslan et al. 2011; Wong et al. 2009a; Xagoraraki et al. 2007). Enteroviruses and rotaviruses have also been detected at some beaches, but two studies involving noroviruses failed to detect them.

Viruses are also found in sediments. When microorganisms enter the natural water, some of them adsorb on the surface of particles that can settle or re-suspend into the water column, since adsorption may be reversible. Re-suspension of enteric viruses in waters impacted by fecal contamination could pose a potential risk to human health (De Flora et al. 1975). Ferguson et al. (1996) suspected that sediments can act as reservoirs for enteric viruses. They took samples from an urban estuary and detected viruses primarily in water and top sediment, whereas no viruses were found in the bottom sediment.

Human enteric viruses have been found in ground water (Abbaszadegan et al. 2003; Fout et al. 2003; Borchardt et al. 2003b; Lieberman et al. 1995; Davis and Witt 1998). In a nationwide study, samples from 448 groundwater sites in 35 states were analyzed for enteroviruses, rotaviruses, hepatitis A viruses and noroviruses. Viral nucleic acid was present in 31% of samples (Abbaszadegan et al. 2003). Human enteric viruses (enteroviruses, hepatitis A viruses, Norwalk viruses, reoviruses or rotaviruses) were detected in 16% of 29 groundwater sites sampled over one year (Fout et al. 2003). Borchardt et al. (2003b) tested 50 private household wells in Wisconsin four times per year and found that four wells (8%) were positive for hepatitis A viruses or rotaviruses, noroviruses and enteroviruses. In an earlier study (Lieberman et al. 1995) in which 30 public water supply wells were examined, the
authors reported that 24% of the samples were positive for culturable viruses. Also, the US Geological Survey (Davis and Witt 1998) reported about 8% of wells positive for culturable human viruses.

Viruses and other microorganisms can survive for several months in soil and groundwater when temperatures are low and soils are moist (Yates et al. 1985; Jansons et al. 1989; Straub et al. 1993; Robertson and Edberg 1997), increasing risk due to groundwater contamination. Presumably, most microbial transport occurs in saturated soil (Jamieson et al. 2002; Powelson and Mills 1998) or by preferential flow (Shipitalo and Gibbs 2000; Mawdsley et al. 1995). Penetration of viruses to depths as great as 67 m (220 ft) and horizontal migration as far as 408 m (1,339 ft) in glacial till and 1,600 m (5,240 ft) in fractured limestone have been reported (Keswick and Gerba 1980; Robertson and Edberg 1997).

1.2.4. Virus survival in the environment

Type of soil, particle size distribution, clay composition, soil organic content, presence of dissolved or colloidal organic carbon, solution chemistry, metal oxides, degree of saturation of the solid media, ionic strength, temperature, pH, light, presence of air-water interfaces, and biological factors are primary factors influencing virus survival and transport in the environment (Gerba 2007; Gerba et al. 1975; Gerba and Bitton 1984; Sobsey et al. 1986; Yates and Yates 1988; Gerba and Rose 1990; Schijven and Hassanizadeh 2000; Jin and Flury 2002; Zhuang and Jin 2003). In water, virus survival mainly depends on temperature, exposure to UV and presence of microbiological flora (Bosch 2006). In seawater at 15°C, polio and adenovirus 40 and 41 can survive for many days. Reduction of 3 logs, 1.4 and 1.6 logs, respectively, were observed after 28 days (Enriquez 1995). In fresh water, human enteroviruses can survive for several weeks. For instance, coxsackievirus B3, echovirus 7 and
poliovirus 1 can be inactivated by 6.5-7 logs over 8 weeks at 22°C, and 4-5 logs over 12 weeks at 1°C (Hurst 1989). In groundwater, the presence of indigenous microorganisms is the important feature in inactivation of enteroviruses (Gordon and Toze 2003). Since UV is destructive for viruses, exposure to UV light or sunlight can enhance virus inactivation in the environment. For example, to achieve inactivation rate of 99% for poliovirus without UV light in marine water, 52 days were needed, while in the presence of sunlight only 21 days were required (Rzezutka and Cook 2004).

1.2.5. Virus transport in the environment

Batch experiments have been used to investigate the factors affecting virus-soil sorption behavior. Jin and Flury (2002) summarized the batch studies done over the previous 20 years. Bacteriophage indicators, and in some cases enteroviruses, were used, and most such studies focused on the effect of pH and ionic strength of the solution, the presence of compounds that compete for binding sites, isoelectric point (IEP) and hydrophobicity of the bacteriophage, and properties of the sorbent. The sorbents used in these studies were mostly soil (sand, silt and clay) and activated carbon. The Freundlich isotherm model \( C_S = K_F C_L^{1/n} \) where, \( C_S \) is the quantity of virus sorbed per unit mass of soil; \( C_L \) is the concentration of virus remaining in the liquid phase; \( K_F \) is the Freundlich constant; \( 1/n \) is a constant) has been used to describe sorption (Drewry and Eliassen 1968; Bitton et al. 1976; Burge and Enkiri 1978; Gerba and Lance 1978; Moore et al. 1981; Jin et al. 1997; Bales et al. 1991; Powelson and Gerba 1994; Thompson et al. 1998; Powell et al. 2000), and studies have determined that (i) clayey soils have higher virus sorption capacity, (ii) an increase in cation concentration in solution can increase virus sorption and (iii) pH affects virus sorption. Burge and Enkiri (1978) found a negative correlation between virus and soil pH, since the virus particles were more positively charged when the soil pH was low, and more readily sorbed on negatively charged soil
surfaces. The presence of organic matter (OM) enhances virus transport (Bixby et al. 1979; Moore et al. 1981; Fuhs et al. 1985; Powelson et al. 1991; Pieper et al. 1997; Zhuang and Jin 2003; Bradford et al. 2006) by competing with virus particles for binding sites and thickening the electrical double layer on sorbent and the virus particles (Cao et al. 2010).

Virus size and surface properties, such as isoelectric point (IEP) and hydrophobicity, play major roles in controlling virus sorption and transport. The size and IEP of selected viruses are summarized in Table 1-2. IEP is the pH at which the virus particle has a net neutral charge. Virus particles exhibit a positive charge when the pH of a solution is below the IEP of virus, and a net negative charge at pH greater than IEP (Vega 2006). IEP has been suggested as the dominant factor controlling virus adsorption during transport through sandy soils (Dowd et al. 1998). However, Dowd et al. (1998) also found that isoelectric points of bacteriophage larger than 60nm did not affect sorption to soil and that bacteriophage size was the overriding determinant of virus sorption.

Zerda et al. (1985) observed that all viruses adsorbed to negatively charged surfaces at pH less than their respective IEP, while viruses would exclusively adsorb to positively charged surfaces at pH greater than IEP. When pH was close to the IPE, viruses adsorbed to all types of silica, although to a lesser extent. Herath et al. (1999) reported that the highest removal for coliphage during microfiltration was achieved near the coliphage’s IEP. Nwachuku and Gerba (2004) suggested that low IEP typically makes microorganisms resistant to water treatment.

Other parameters that control virus sorption and transport are zeta potential and hydrophobicity of the virus. Zeta potential refers to “the mean electrostatic potential at the closest separation between a small ion and the charged macroparticle” (Yu et al. 2004), and it is related to the stability of colloidal dispersions. The zeta potential is a function of solution pH since viruses become more negatively charged in higher pH waters (Liu et al. 2009; Gitis et al. 2002). Ionic strength can also affect zeta potential. It has been reported that in
NaHCO₃-NaCl solution (pH = 7), the zeta potential of poliovirus is -1.8±0.3mV and -5.9±0.9mV at ionic strengths of 0.3M and 0.2M, respectively (Murray and Parks 1980). At low zeta potentials, viruses tend to coagulate or flocculate, and thus their transport may be retarded.

Hydrophobicity is another important surface property. It has been suggested that viruses with a lipid envelope are generally hydrophobic, while viruses without a lipid envelope tend to be hydrophilic (Vidaver et al. 1973). Kinoshita et al. (1993) compared PRD-1 and MS2 phages and suggested that the less hydrophilic phage (MS-2) acted conservatively and was not removed in sand columns at pH 5.7-8.0. Farrah et al. (1981) reported that hydrophobic interactions are the dominant determinant of virus attachment during flow through porous media so that hydrophobic effects are of primary importance to virus removal from water (Powelson 1990; Murray 1980).

Numerous studies have used isotherm approaches to evaluate the factors that affect desorption behavior of chemical compounds, but desorption isotherms have not been developed for viruses or viral indicators. Chetochine et al. (2006) found that after a series of 17 extractions (25ml sample volume with 2% biosolids) from solid media, 10³ PFU of bacteriophage MS2 remained in the pelletized solid, but almost no MS2 were in the supernatant. Also, it has been reported that enteroviruses (Gerba 1981; Pancorbo et al. 1981) and coliphage (Gerba et al. 1978) attach strongly to solid phases and are difficult to elute from sludge.

1.3. Detection Methods

Traditionally, cell culture has been the method used for virus detection. In this method, infected cell cultures undergo morphological changes called cytopathic effects (CPEs) that are observed microscopically. The method is labor intensive and some viruses do not exhibit
CPEs. Also traditionally, plaque assays are used to detect phages. In this method, a confluent monolayer of host cells is infected with the virus, and the infected area will create a plaque. By counting the number of plaques, virus concentration can be determined and represented in terms of plaque forming units.

PCR is emerging very rapidly as a method for virus detection in environmental samples. Compared to cell culture, the main advantages of PCR methods for virus detection include fast results, high specificity and sensitivity, and the ability to detect difficult to culture or non-culturable viruses such as adenovirus 40/41 and noroviruses. The main disadvantage of PCR methods is that they do not provide a measure of infectivity. There are also problems associated with detection limits and environmental inhibition. Microarrays can also be used for the detection of viruses. Hundreds or thousands of genes can be studied simultaneously using DNA microarrays, and the procedure is relatively fast.

Conventional PCR can amplify and detect virus-specific DNA sequences in the presence of DNA from many other sources. Gel electrophoresis is needed afterward in order to visualize the results. Normally conventional PCR is not a quantitative assay, but quantitative results can be generated by using dilutions and the most probable number (MPN) method. Reverse transcription PCR is used to produce a complementary strand (cDNA) for RNA viruses such as enteroviruses and noroviruses. Nested PCR generally has two sets of primers, one set nested within the nucleic acid defined by the second primer pair. An amplicon is generated by the outer primers, while the target sequence of DNA is amplified by inner primers. In Multiplex PCR, multiple DNA sequences are targeted simultaneously. Real-time PCR is a quantitative assay in which target sequences are simultaneously amplified and quantified. In addition to primers, a set of probes with attached dyes is involved in real-time PCR. During amplification, the dyes are released from the probes and fluoresce. The fluorescence signal can be detected and, using a standard curve, the number of viral genome copies is quantified. When combined with cell culture, PCR can be employed to determine
the infectivity of viruses using a procedure called integrated cell culture PCR (ICC-PCR).

A simplified schematic of virus detection methods in environmental media is shown in Figure 1-2. Sample collection and pre-treatment is a critical aspect of all environmental virology methods and pre-treatment methods are also shown in Figure 1-2. Virus concentration in natural water bodies is usually low, and pre-concentration of viruses is often the most important step for effective detection. The technique most commonly used to concentrate viruses from water samples is the virus adsorption-elution microporous filter method, or VIRADEL. The filters for VIRADEL can be electropositive or electronegative. When using negative filters, adjustment of cationic salt concentration and pH is needed prior to sample processing. Electropositive filters do not require pre-treatment. The most commonly used electropositive filters are 1MDS filters and NanoCeram cartridge filters.

After filtration, an elution step follows. The purpose of elution is to release the viruses captured by the cartridge filters (water samples) or to isolate viruses from sludge/sediment grab samples. The elution procedure for cartridge filter samples follows EPA’s virus adsorption-elution VIRADEL method (USEPA 2001a). Briefly, the filters are backwashed with beef extract solution. Elutes containing viruses are flocculated by lowering pH. Flocs are isolated by centrifugation and re-suspended in sodium phosphate. Following neutralization and centrifugation, supernatants containing viruses are separated. Sludge, sediment or biosolids samples for viral analysis are eluted using ASTM Method D4994-89. The samples are mixed with beef extract, and pH is adjusted to about 3.5 to promote flocculation. Pellets are collected after centrifugation and re-suspended in phosphate buffered saline. pH is neutralized, before eluted samples are passed through membrane filters.
1.4. Fate of Viruses during Water Treatment

1.4.1. Fate of viruses during full-scale water treatment

Since enteric viruses are transmitted mostly by the fecal – oral route, water treatment provides a critical barrier to the release of viruses in potable water. According to the EPA National Primary Drinking Water Standards, enteric viruses must be removed or inactivated by 4 logs (99.99%) during water treatment from surface waters (USEPA 2001b). However, on the several occasions, viruses have been released by drinking water utilities. In general, even though most water treatment plants can achieve more than 4 log virus reduction (Payment et al. 1993, Paymet et al. 1985), viruses have been detected in finished water. A possible explanation for those observations lies in the susceptibility of viruses to chlorine inactivation (Payment et al. 1985). Coxsackieviruses are more resistant to chlorination than polioviruses or reoviruses. To achieve 4-log inactivation for coxsackieviruses, 40 minutes contact time is generally needed compared to 5 minutes for reoviruses (Payment et al. 1985). Virus survival in finished water also results from operational difficulties that lead to violation of treatment objectives related to turbidity and chlorine residual. Inadequate floc formation, floc breakdown, and filter overloading can lead to ineffective disinfection and virus survival (Keswick et al. 1984). For example, Keswick et al. (1984) detected rotaviruses or enteroviruses in effluent from a conventional drinking water treatment plant. They reported that 25 - 93% enteric viruses were removed during the dry season, while the removal efficiency was only 0 - 43% during the rainy season. When the quality of water declined, the removal of viruses decreased as well. One of the possible reasons was adsorption to the particles that were not removed during clarification and filtration, and protected the viruses from final chlorination.
1.4.2. Virus inactivation

Commonly used methods for drinking water disinfection are chlorination, ozonation, and UV irradiation. Chlorine achieves inactivation/destruction by oxidizing cellular materials of target microorganisms. This technique is cheap and well-established, but carcinogenic chlorination by-products may be formed under certain conditions (USEPA 1999b). Chlorine dose and contact time are keys to virus removal. Higher dose and longer contact time generally produce higher removal efficiencies. For example, Abad et al. (1994) reported that the log inactivation of adenoviruses rose from 2.5 to 3.2 by doubling the dose of free chlorine. Shin and Sobsey (2008) reported that inactivation of poliovirus was enhanced with higher dose of chlorine, even though the contact time was shorter. A series of experiments carried out by Thurston-Enriquez et al. (2003a) showed that the virus removal (adenovirus 40 and poliovirus 1) was directly related to contact time. Similar results were obtained by Thurston-Enriquez et al. (2005b) using chlorine dioxide. The pH for disinfection usually ranges from 6 to 8. Data from Alvarez and O’Brien (1982) indicate that significantly higher removal efficiencies for polioviruses can be obtained at pH 10 compared to pH 6, but the effect of pH on virus inactivation during disinfection remains uncertain and may vary between viruses.

Ozone is more effective than chlorine for virus disinfection but provides no residual for protection against regrowth during water distribution. It is also very reactive and corrosive, and the cost of ozonation can be high. In addition, the presence of bromide ion in the raw water may lead to formation of brominated by-products (USEPA 1999b). The mechanism of ozone disinfection involves destruction of the cell structures (cell wall, nucleic acids, etc.) by direct oxidation or reactions involving radical intermediates that are produced during ozone decomposition (USEPA 1999b; USEPA 1999c). Similar to chlorination, higher dose of ozone and longer contact time generally result in better performance for virus inactivation. For instance, the log removal of poliovirus doubled when the ozone dose increased from 0.4mg/l
to 1.24mg/l (Katzenelson et al. 1979), while adenovirus removal slightly increased as a consequence of longer contact time (Thurston-Enriquez et al. 2005a). Temperature seems to be another important parameter, and lower temperature tended to facilitate virus inactivation (Herbold et al. 1989). No uniform relationship was found between pH and inactivation efficiency.

UV irradiation can penetrate cell structures, damage genetic materials and interfere with cell reproduction. It involves no chemical addition, and thus no residual or chemical intermediates will be formed and released to the environment. Disinfection with UV may depend on UV lamp type. For instance, medium-pressure UV lamps can achieve higher inactivation rates compared to low-pressure lamps at the same total intensity (Eischeid et al. 2009, Guo et al. 2010, Linden et al. 2007; Linden et al. 2009). Higher UV dose can steadily increase inactivation of a variety of viruses, such as echovirus, coxsackievirus, poliovirus, and adenovirus (Gerba et al. 2002b; Ko et al. 2005; Thompson et al. 2003; Simonet and Gantzer 2006). Some viruses can’t be inactivated by UV very effectively, especially when the UV dose is low. For example, it is widely known that human adenoviruses are very resistant to UV (Ballester and Malley 2004; Chang et al. 1985; Eischeid et al. 2009; Gerba et al. 2002b; Ko et al. 2005; Nwachuku et al. 2005; Thurston-Enriquez et al. 2003b).

1.5. Fate of Viruses in Wastewater Treatment Systems

1.5.1. Virus removal in full-scale wastewater utilities

et al. 2011; Simmons and Xagoraraki 2011). Membrane bioreactors (MBRs) are expected to provide higher quality effluents. This technology involves the combination of the activated sludge biological treatment with biomass separation by membrane filtration in a submerged or side-stream configuration. When well designed and operated, MBRs can consistently achieve efficient removals of suspended solids (Vaid et al. 1991), chemical oxygen demand (Pankhania et al. 1994; Beaubien et al. 1996), biochemical oxygen demand (Kishino et al. 1996), nitrogen (Kishino et al. 1996; Gujer et al. 1999), phosphorus (Schaum et al. 2005) and coliform bacteria (Van der Roest et al. 2002). Under optimal conditions, MBR systems can also reliably remove various viruses and phages (Table 1-3). For example, Kuo et al. (2010) reported 4.1-5.6 log removals for human adenoviruses, while Simmons et al. (2011) reported that removal efficiencies could reach 6.3, 6.8, and 4.8 logs for human adenoviruses, enteroviruses, and noroviruses respectively. Da Silva et al. (2007) obtained high removal efficiencies for noroviruses in a full-scale MBR system, but their data also suggest that virus removals were inconsistent.

Removal of viruses in full-scale conventional wastewater treatment plants (WWTP) and full-scale MBR systems are compared in Figures 1-3 through 1-6. Overall, full-scale MBR plants achieved higher virus removals. Adenovirus removal in WWTPs prior to disinfection (Table 1-3 and Figure 1-3) ranged from 1.02 logs to 4.08 logs (Haramoto et al. 2007; Hewitt et al. 2011). Katayama et al. (2008) reported that in WWTPs, the virus removal due to disinfection was 1.65 logs on average. Adenovirus removals in advanced treatment systems such as MBRs were significantly higher – ranging from 3.4 logs to 6.3 logs (Kuo et al. 2010; Simmons et al. 2011; Simmons and Xagoraraki 2011).

Figure 1-4 shows a summary of enterovirus removals in full-scale WWTPs. In conventionally treated wastewater prior to disinfection, virus removals ranged from 0.7 logs to 2.4 logs (Lodder et al. 2005; Costan-Longrades et al. 2008; Hewitt et al. 2011). Conventional plants with disinfection produced higher virus removals: up to 5.23 logs

As shown in Figure 1-5, reduction of norovirus I in conventional WWTPs without disinfection was less than 1.4 logs (Hewitt et al. 2011; Nordgren et al. 2009). WWTPs with disinfection performed slightly better with log removals from 0.95 to 2.69 (Katayama et al. 2008). In MBR plants without disinfection the removal of norovirus I was up to 5.5 logs (da Silva et al. 2007). Norovirus II removals in full-scale WWTPs are summarized in Figure 1-6. The highest virus reduction in a conventional WWTP without disinfection was 1.2 logs (Hewitt et al. 2011; Nordgren et al. 2009), whereas, with disinfection, virus removal ranged from 1.3 to 3 logs (Katayama et al. 2008). For MBR plants, removals in the range of 2.3 logs to 4.9 logs were observed (da Silva et al. 2007; Simmons et al. 2011).

1.5.2. Virus removals in bench and pilot-scale MBR systems

Bench and pilot-scale MBR studies have been performed to describe virus removal. MS-2 coliphage appears to be the most common virus used in bench scale MBR studies. It is a single-stranded RNA virus, with icosahedral shape, small size (20 nm to 25 nm), and low IEP (3.9) (Zerda 1982) and relative hydrophobicity (Oh et al. 2007). These characteristics are similar to some pathogenic human viruses found in water and wastewater such as hepatitis A virus and poliovirus (Fiksdal et al. 2006), and thus make MS-2 a good indicator and surrogate for virus studies with membrane systems (Shang et al. 2005; Comerton et al. 2005). Both indigenous and lab-cultured MS-2 phages were used in these studies, and quantification was done by plaque assay. T4 coliphage has also been used in bench-scale MBR studies since it is similar to adenoviruses, reoviruses, rotaviruses (Zheng and Liu 2007), and coronaviruses (Lv et al. 2006). Even though the size and IEP of phages are similar to those of some enteric
viruses, their removal and transport do not necessarily relate to those of enteric viruses in wastewater systems, and therefore further research is needed.

As shown in Table 1-4, bench and pilot scale MBRs can achieve high removals of coliphages. Five potential mechanisms for virus removal were suggested (Ravindran et al. 2009): (1) rejection of virus by a gel layer consisting of natural organic matter; (2) rejection by a layer of microbial biomass; (3) rejection due to internal pore blocking by natural organic matter; (4) adsorption on the surface of membranes and bio-particles; and (5) combinations of these mechanisms.

MBR systems with higher hydraulic retention times (HRT) and lower solids retention times (SRT) appear to be more efficient in removing viruses (Wu et al. 2010). Madaeni et al. (1995) suggested that the presence of biomass, low trans-membrane pressure and stirring enhance virus removal during the membrane filtration process.

Membrane pore size may be an important determinant of virus removal efficiency. Membranes with smaller pore sizes tend to achieve higher removal for viruses, but not always (Figure 1-7). Madaeni et al. (1995) reported that hydrophobic PVDF membrane (pore size = 0.22 µm) could remove about 99% of poliovirus, while ultrafiltration membranes with pore sizes smaller than the virus achieved complete rejection. However, it has been observed that in MBR systems with a range of membrane pore sizes (0.03-0.1 µm) indigenous MS-2 was not detectable in the effluent, and removal mechanisms other than straining may exist (Hirani et al. 2010). According to Zheng and Liu (2007) and Zheng et al. (2005), there was no significant difference in virus removal efficiency using membranes with 0.1 µm and 0.22 µm pore sizes, whereas Lv et al. (2006) indicated that a 0.1 um membrane was more effective than a comparable 0.22 µm membrane. Fiksdal et al. (2006) reported that phages were poorly removed during MBR treatment without pre-coagulation / flocculation, even using ultrafiltration membranes.
1.5.3. Viruses in biosolids

Most wastewater virus studies report numbers of viruses in effluent or removal efficiencies that reflect virus concentrations in influent and effluent. Since viruses tend to attach to solid surfaces, most viruses that survive wastewater treatment are likely associated with waste activated sludge and may be present in biosolids. In the US, approximately 5.6 million dry tons of biosolids are generated annually, 60 percent of which are land applied as a soil amendment (NRC 2002). The US EPA divides biosolids into two classes: class A or pathogen-free biosolids, and class B biosolids, which may have some pathogens such as human adenovirus (USEPA 2003). Different treatment methods can be used to produce class A biosolids, and the removal of viruses is established using bacterial indicators such as fecal coliforms (USEPA 2003). Class A biosolids are sold directly to the public for lawn and garden use and should not contain detectable pathogens. Class B biosolids can be applied on agricultural and forest lands as fertilizers. Monitoring for enteroviruses in biosolids is now encouraged but not required by the EPA, and reports of enteric viruses in sludge and biosolids are limited. Table 1-5 indicates that class B biosolids contain potentially infectious viruses. Using integrated cell culture-PCR, relatively large numbers of viable viruses have been detected in class B biosolids (Wong et al. 2010).

1.5.4. Bacterial viruses (phages) in wastewater

Bacteriophages, or phages, are viruses that infect bacteria. All contain nucleic acid surrounded by a protein coat that enables them to stick to bacterial cell envelopes. When attached, they inject DNA into the host bacteria. It is suggested that phage abundance in activated sludge at wastewater treatment plants is higher than any other environment (Shapiro et al. 2011; Rosenberg et al. 2010; Wu and Liu 2009; Otawa et al. 2007). In activated sludge the phage-to-bacterial-cell-ratio is approximately 10:1 (Rosenberg et al. 2010). Thus,
important phage-bacteria interactions may take place during wastewater treatment.

For example, bacteriophages may play a major role in bacterial evolution by facilitating the transfer of antibiotic resistance genes (ARG) or other genes to new bacterial hosts via transduction (Mazaheri Nezhad Fard et al. 2010; Canchaya et al. 2004; Boyd and Brussow 2002). Horizontal gene transfer is the movement of genetic material among bacterial species without cell division. It provides an important mechanism for accelerating the dispersal of ARGs in the environment (Colomer-Lluch et al. 2011; Baquero et al. 2008; Sander et al. 2001). In recent years, there have been many efforts to study gene transfer mechanisms that are responsible for the spread of antibiotic resistance among bacteria. Transformation is the direct uptake of naked DNA from the cell surroundings. Conjugation is the transfer of DNA mediated by a conjugative or mobilizable genetic element (plasmids or transposons). It requires cell to cell contact and long fragments of DNA can be transferred through this mechanism. The transfer of DNA mediated by bacteriophage is known as transduction. Very little information is available regarding phage-mediated transduction (Colomer-Lluch et al. 2011; Sander et al. 2001). Only a small fraction of general transducing bacteriophages have been characterized so far, and only a few studies have looked for antibiotic resistance genes in bacteriophage isolated from wastewater treatment plants or surface waters impacted by the discharge of treated wastewater (Colomer-Lluch et al. 2011; Mazaheri Nezhad Fard et al. 2010; Parsley et al. 2010; Muniesa et al. 2004; Prescott 2004). For example, Colomer-Lluch et al. (2011) highlighted the potential role of phages in the spread of β lactamase genes in urban sewage and river water samples and found that phages may act as reservoirs for the spread of ARGs in the environment. Another study was done on enterococcal bacteriophages that play a role in successful transfer of antibiotic resistant genes for tetracycline and gentamicin resistances between the same and different enterococcal species (Mazaheri Nezhad Fard et al. 2011).

There are other ways in which bacteriophages are important in wastewater treatment
systems. As mentioned previously, bacteriophages infect bacteria; thus, they can control bacterial community structure. Researchers have proposed the use of phages during wastewater treatment to improve effluent and sludge characteristics (Withey et al. 2005). Using phages, it may be possible to improve wastewater treatment performance by, for example, controlling foam in activated sludge treatment, attacking pathogenic bacteria, and reducing the competition between insignificant (from the perspective of waste conversion) and critically important bacterial populations. However, such modifications require a more complete understanding of wastewater microbial community dynamics including phage-dependent interactions (Withey et al. 2005). Next generation sequencing and metagenomics are powerful tools that can provide information about phages and their significance.

1.6. Viral Risk Assessment

Quantitative viral risk assessment (QVRA) studies have been published for wastewater systems. Exposure to human enteric viruses from wastewater-related products (post-disinfected effluents and sludge) occur during recreational activities in surface waters, sludge handling, land application of biosolids, ingestion of untreated surface and ground waters and other exposure pathways resulting in inhalation and ingestion-related health risks (Haas 1983; Lapen et al. 2008; Viau and Peccia 2009).

In general, quantitative microbial risk assessment includes hazard identification, exposure assessment (determination of exposure routes, pathogen dose, and exposure parameters), determination of dose-response relationships, and risk characterization. Dose-response assessment characterizes the correlation between probability of infection and exposure to viruses. The number of viruses ingested is estimated by Equation 1 (Haas et al. 1999). The exponential model (Equation 2) and beta-Poisson model (Equation 3) have been
used extensively to represent dose-response relationships (Table 1-6) and estimate the probability of infection. As \( \alpha \) increases, the beta-Poisson model approaches the exponential model (Haas et al. 1999).

\[
N = C \times \frac{1}{R} \times I \times 10^{-DR} \times V \quad \text{Equation [1]}
\]

\[
P_{i/day} = 1 - \exp(-rN) \quad \text{Equation [2]}
\]

\[
P_{i/day} = 1 - (1 + \frac{N}{\beta})^{-\alpha} \quad \text{Equation [3]}
\]

Where, \( N \) is number of viruses ingested; \( C \) is the concentration of viruses; \( R \) is the efficiency of recovery method; \( I \) is the fraction of detected viruses that are capable of infection; \( DR \) is the removal or inactivation efficiency of the treatment process. For recreational water, \( DR \) is equal to 0, since no treatment is applied; \( V \) is the daily volume of recreational water consumed by individuals that are exposed to the water. \( P_{i/day} \) is the probability of becoming infected, \( \alpha \) and \( \beta \) are two parameters for Poisson distribution.

Several QVRA studies have been performed using virus indicators such as bacteriophage and viruses in the environment (Haas 1983; Regli et al. 1991; Dowd et al. 2000; Gerba et al. 2002a; Eisenberg et al. 2006 and 2008). QVRA studies have generally used culture-based virus measurements to estimate ingested viral dose, assuming that a single virus can be used to represent total human enteric viruses (Haas 1983; Regli et al. 1991; Gerba et al. 2002a; Eisenberg et al. 2008). For example, during biosolids-based QVRA studies (Gerba et al. 2002a; Eisenberg et al. 2008) the total concentration of biosolids-associated viruses was represented in terms of the measured concentrations of rotaviruses or echovirus-12 to calculate risk estimates. Other QVRA studies have used viral genomic copies (GCs) measured via PCR to estimate ingested dose of a specific virus type, with or without adjustments to convert GCs to infectious virus concentrations (Masago et al. 2006; Teunis et al. 2008; Schoen and Ashbolt 2010). Masago et al. (2006) assumed that the total GC measurement of noroviruses represents the infectious concentration of noroviruses to assess
risk from ingestion of water. Teunis et al. (2008) and Schoen and Ashbolt (2010) assumed that the infectious concentration of noroviruses is half the measured number of norovirus GCs in order to estimate the risk of infection from ingestion of water during recreational activities. Viau and Peccia (2009) used a similar approach for converting adenovirus GCs to infectious adenovirus concentration for estimating risks of inhalation of bioaerosols (0.1% conversion factor calculated using data for primary effluent samples obtained from He and Jiang (2005)). The use of different assumptions for relating GCs to infectious virus concentrations (infectivity ratios) in QVRA studies poses a consistent and significant uncertainty in estimates of infectious viral doses.

The risk of virus infection from applied biosolids appears to be low. For example, Gerba et al. (2002a) estimated that such risk was less than $10^{-4}$ (1 out of 10,000 risk of infection). Kumar et al. (2012) reported that the viral infection risk of soil ingestion of biosolids was greater than $10^{-4}$, based on the data obtained from both cell culture and genomic methods. At recreational beaches, Wong et al. (2009a) estimated the daily risk of viral infection ranged from 0.2 to 2.4 per 1000 swimmers.

### 1.7. Summary and Conclusions

Occurrence of human pathogenic viruses in environmental waters (i.e. surface waters, groundwater, drinking water, recreational water, and wastewater) raises concerns regarding the possibility of human exposure and waterborne infections. Commonly observed waterborne viruses include adenoviruses, enteroviruses, noroviruses, and rotaviruses. Much attention has been given recently to human adenoviruses due to related health implications that range from diarrhea to death.

Viruses are the smallest of all microorganisms, and their size facilitates transport in environmental media. In addition, viruses have very low die-off rates and low infectivity
doses, increasing concern over outbreaks of disease related to waterborne or sludge-related virus exposures. The ability to detect waterborne viruses effectively is the basis for microbial risk assessment and management of water resources for the protection of public health. However, precise detection, quantification, and infectivity determination for viruses remain challenging.

Wastewater is a major source of viruses in the environment. Especially when water reuse is contemplated, appropriate technologies must be practiced that yield a virus-free effluent. Membrane bioreactors have been shown to reduce numbers of viruses more effectively than conventional activated sludge facilities. Even though advances in wastewater treatment technology in recent decades have greatly reduced waterborne disease, human enteric viruses are still detected in the effluents of state-of-the-art wastewater treatment plants worldwide, including those with membrane bioreactors.

Viruses have also been observed in the effluent of conventional drinking water utilities. In drinking water treatment, inactivation of resistant viruses poses a challenge, particularly for small-scale or point-of-use systems. For example, adenoviruses are very resistant to UV disinfection.

Overall, the presence of viruses in water and wastewater is a difficult problem for environmental engineers, due to the small sizes, prevalence, infectivity, and resistance of viruses to disinfection. Here, we briefly described virus survival and behavior in the environment and reviewed both virus-associated diseases and their transmission pathways. Environmental engineers should be aware that wastewater treatment plants are not able to remove many viruses from wastewater. Viruses discharged from drinking water treatment plants due to technical and management deficiencies may increase human exposure and disease. The knowledge summarized provides basic information needed to make decisions for efficient water and wastewater management and reduction of risk arising from human exposure to viruses.
APPENDIX
<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Classification</th>
<th>CCL 1</th>
<th>CCL 2</th>
<th>CCL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviruses</td>
<td>Adenoviridae</td>
<td>Group I (double strand DNA)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Enteroviruses*</td>
<td>Picornaviridae</td>
<td>Group IV (positive single-stranded RNA)</td>
<td>---</td>
<td>---</td>
<td>Yes</td>
</tr>
<tr>
<td>Coxsackieviruses</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>---</td>
</tr>
<tr>
<td>Echoviruses</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>---</td>
</tr>
<tr>
<td>Hepatitis A viruses</td>
<td></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>Yes</td>
</tr>
<tr>
<td>Caliciviruses</td>
<td>Caliciviridae</td>
<td>Group IV (positive single-stranded RNA)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Polioviruses, coxsackieviruses, and echoviruses are generally referred to as enteroviruses.
Table 1-2. Summary of Virus Surface Properties Affecting Sorptive Removal from Water

<table>
<thead>
<tr>
<th>Virus (1)</th>
<th>Virion Size (nm)</th>
<th>Isoelectric Point (IEP)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus</td>
<td>22-30</td>
<td>4.0 - 6.4</td>
<td>Minor, 1987; Gre and Pavelic, 2004; Murry and Parks, 1980; Butler et al., 1985; Zerda and Gerba, 1984</td>
</tr>
<tr>
<td>Coxsackieviruses</td>
<td>4.75 - 6.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echoviruses</td>
<td>4.0 - 6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A viruses</td>
<td>27-28</td>
<td>2.8</td>
<td>Minor, 1987; Nasser et al., 1992</td>
</tr>
<tr>
<td>Caliciviruses</td>
<td>30-40</td>
<td>5.5 - 6.0 (2)</td>
<td>Carter et al., 1987; Goodridge et al., 2004</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>70-140</td>
<td>3.5 - 4.5</td>
<td>Nermut, 1987; Trilisky and Lenhoff, 2007; Wong et al., 2012b; Stewart, 1991</td>
</tr>
</tbody>
</table>

(1) All viruses in CCL are non-enveloped and icosahedral in shape.  
(2) For Norwalk virus (a member of noroviruses).
## Table 1-3. Virus Removal in Full-Scale Membrane Bioreactors

<table>
<thead>
<tr>
<th>Membrane pore size</th>
<th>Virus (source)</th>
<th>Detection methods</th>
<th>Removal efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 µm</td>
<td>F-specific Coliphage</td>
<td>Plaque assay</td>
<td>6.0 logs</td>
<td>Zanetti et al., 2010</td>
</tr>
<tr>
<td>0.4 µm</td>
<td>Somatic Coliphage</td>
<td>Plaque assay</td>
<td>4.0 logs</td>
<td>Zanetti et al., 2010</td>
</tr>
<tr>
<td>0.1 µm</td>
<td>HAdV</td>
<td>qPCR</td>
<td>4.1-5.6 logs</td>
<td>Kuo et al., 2010</td>
</tr>
<tr>
<td>0.4 µm</td>
<td>Norovirus I</td>
<td>qPCR</td>
<td>0-5.3 logs*</td>
<td>da Silva et al., 2007</td>
</tr>
<tr>
<td>0.4 µm</td>
<td>Norovirus II</td>
<td>qPCR</td>
<td>0-5.5 logs*</td>
<td>da Silva et al., 2007</td>
</tr>
<tr>
<td>NA</td>
<td>HAdV</td>
<td>qPCR</td>
<td>3.4-4.5 logs+</td>
<td>Simmons and Xagoraraki, 2011</td>
</tr>
<tr>
<td>0.1 µm</td>
<td>HAdV</td>
<td>qPCR</td>
<td>4.1-6.3 logs</td>
<td>Simmons et al., 2011</td>
</tr>
<tr>
<td>0.1 µm</td>
<td>Enterovirus</td>
<td>qPCR</td>
<td>4.1-6.8 logs</td>
<td>Simmons et al., 2011</td>
</tr>
<tr>
<td>0.1 µm</td>
<td>Norovirus (II)</td>
<td>qPCR</td>
<td>3.5-4.8 logs</td>
<td>Simmons et al., 2011</td>
</tr>
</tbody>
</table>

* Obtained from graphs
<table>
<thead>
<tr>
<th>Scale</th>
<th>Membrane pore size</th>
<th>Virus (source)</th>
<th>Detection methods</th>
<th>Removal efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench</td>
<td>0.4 µm</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>0.4-2.5 logs</td>
<td>Shang et al., 2005</td>
</tr>
<tr>
<td>Bench</td>
<td>0.2 µm</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>Average 6.7 logs</td>
<td>Fiksdal et al., 2006</td>
</tr>
<tr>
<td>Bench</td>
<td>0.45 µm</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>0.31-1.5 logs</td>
<td>Oh et al., 2007</td>
</tr>
<tr>
<td>Bench</td>
<td>UF and NF</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>2 logs for UF</td>
<td>Hu et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 logs for NF</td>
<td></td>
</tr>
<tr>
<td>Bench</td>
<td>0.1 and 0.22µm</td>
<td>T4 Coliphage</td>
<td>Plaque assay</td>
<td>5-8 logs for 0.1 µm</td>
<td>Lv et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5-6 logs for 0.22µm</td>
<td></td>
</tr>
<tr>
<td>Bench</td>
<td>0.1 and 0.22µm</td>
<td>T4 Coliphage</td>
<td>Plaque assay</td>
<td>5.5 logs</td>
<td>Zheng and Liu., 2007</td>
</tr>
<tr>
<td>Bench</td>
<td>0.4µm</td>
<td>Somatic Coliphage</td>
<td>Plaque assay</td>
<td>6 logs</td>
<td>Zheng et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5-2.5 logs</td>
<td>Wu et al., 2010</td>
</tr>
<tr>
<td>Pilot</td>
<td>300k Da</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>No plaques observed</td>
<td>Cicek et al., 1998</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.04-0.1 µm</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>1.0-4.4 logs</td>
<td>Hirani et al., 2010</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.2 µm</td>
<td>MS-2</td>
<td>Plaque assay</td>
<td>3.8 logs</td>
<td>Ravindran et al., 2009</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.1 µm</td>
<td>Somatic Coliphage</td>
<td>Plaque assay</td>
<td>No plaques observed</td>
<td>Ahn et al., 2001</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.03 µm</td>
<td>Somatic Coliphage</td>
<td>Plaque assay</td>
<td>3.7 logs</td>
<td>Wong et al., 2009b</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.4 µm</td>
<td>F-specific Coliphage</td>
<td>Plaque assay</td>
<td>&gt; 4.0 logs</td>
<td>Tam et al., 2007</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.1µm</td>
<td>F-specific Coliphage</td>
<td>Plaque assay</td>
<td>No plaques observed</td>
<td>Ahn et al., 2001</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.04 µm</td>
<td>Enteric cytopathogenic bovine orphan virus</td>
<td>Plaque assay</td>
<td>Not detectable in effluent</td>
<td>Krauth et al., 1993</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.45 µm</td>
<td>Norovirus</td>
<td>PCR</td>
<td>-0.19 - -0.01</td>
<td>Ottoson et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterovirus</td>
<td></td>
<td>-0.05 - -0.03</td>
<td></td>
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</tbody>
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Table 1-5. Virus occurrence in dewatered sludge and class B biosolids

<table>
<thead>
<tr>
<th>作者</th>
<th>检测方法</th>
<th>病毒</th>
<th>出现平均值</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bofill-Mas et al., 2006</td>
<td>qPCR</td>
<td>腺病毒</td>
<td>$1.1 \times 10^2$ copies/g</td>
</tr>
<tr>
<td>Monpoeho et al., 2001</td>
<td>RT-PCR</td>
<td>肠病毒</td>
<td>$4.8 \times 10^4$ copies/10g</td>
</tr>
<tr>
<td></td>
<td>Cell Culture</td>
<td>腺病毒</td>
<td>$7$ MPNCU*/10g</td>
</tr>
<tr>
<td>Viau and Peccia, 2009</td>
<td>qPCR</td>
<td>腺病毒</td>
<td>$2.5 \times 10^4$ copies/g</td>
</tr>
<tr>
<td></td>
<td>Cell Culture</td>
<td>腺病毒</td>
<td>$1.9 \times 10^6$ copies/g</td>
</tr>
<tr>
<td></td>
<td>qPCR</td>
<td>肠病毒</td>
<td>$2.3 \times 10^5$ copies/g</td>
</tr>
<tr>
<td>Wong et al., 2010</td>
<td>qPCR</td>
<td>腺病毒</td>
<td>$2.5 \times 10^4$ copies/g</td>
</tr>
<tr>
<td></td>
<td>Cell Culture</td>
<td>肠病毒</td>
<td>$1.9 \times 10^5$ copies/g</td>
</tr>
<tr>
<td></td>
<td>Cell Culture</td>
<td>腺病毒</td>
<td>2210 MPN/4 g</td>
</tr>
</tbody>
</table>

**Class B Biosolids**

<table>
<thead>
<tr>
<th>作者</th>
<th>检测方法</th>
<th>病毒</th>
<th>出现平均值</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bofill-Mas et al., 2006</td>
<td>qPCR</td>
<td>腺病毒</td>
<td>$10^3$ copies/g</td>
</tr>
<tr>
<td>Monpoeho et al., 2001</td>
<td>RT-PCR</td>
<td>肠病毒</td>
<td>$1.06 \times 10^4$ copies/10g</td>
</tr>
<tr>
<td></td>
<td>Cell Culture</td>
<td>腺病毒</td>
<td>9 MPNCU/10g</td>
</tr>
<tr>
<td>Monpoeho et al., 2004</td>
<td>RT-PCR</td>
<td>肠病毒</td>
<td>$1.2 \times 10^5$ copies/g</td>
</tr>
<tr>
<td></td>
<td>Cell Culture</td>
<td>腺病毒</td>
<td>38.2 MPNCU/g</td>
</tr>
<tr>
<td>Viau and Peccia, 2009</td>
<td>qPCR</td>
<td>腺病毒</td>
<td>$5 \times 10^5$ copies/g</td>
</tr>
<tr>
<td></td>
<td>qPCR</td>
<td>腺病毒</td>
<td>$7.5 \times 10^5$ copies/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>肠病毒</td>
<td>$1.9 \times 10^6$ copies/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>肠病毒</td>
<td>$5 \times 10^6$ copies/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>肠病毒</td>
<td>$1.5 \times 10^7$ copies/g</td>
</tr>
<tr>
<td>Wong et al., 2010</td>
<td>Cell Culture</td>
<td>腺病毒</td>
<td>480 MPN/4g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>肠病毒</td>
<td>肠病毒</td>
</tr>
</tbody>
</table>

*MPNCU – most-probable-number cytopathogenic units.
### Table 1-6. Dose response models for enteric viruses

<table>
<thead>
<tr>
<th>Waterborne Virus</th>
<th>Exposure</th>
<th>Dose-response Model</th>
<th>Defined Parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteroviruses 68-71</td>
<td>Ingestion</td>
<td>Beta-Poisson</td>
<td>$a = 0.67$, $\beta = 47.9$</td>
<td>Soller et al., 2004</td>
</tr>
<tr>
<td>Poliovirus 1</td>
<td>Ingestion</td>
<td>Exponential</td>
<td>$r = 0.009102$</td>
<td>Regli et al., 1991</td>
</tr>
<tr>
<td>Poliovirus 1</td>
<td>Ingestion</td>
<td>Beta-Poisson</td>
<td>$a = 0.1097$, $\beta = 1524$</td>
<td>Regli et al., 1991</td>
</tr>
<tr>
<td>Poliovirus 3</td>
<td>Ingestion</td>
<td>Beta-Poisson</td>
<td>$a = 0.409$, $\beta = 0.788$</td>
<td>Regli et al., 1991</td>
</tr>
<tr>
<td>Coxsackievirus A21</td>
<td>Inhalation</td>
<td>Exponential</td>
<td>$r = 0.0145$</td>
<td>Haas et al., 1999</td>
</tr>
<tr>
<td>Coxsackievirus B4</td>
<td>Inhalation</td>
<td>Exponential</td>
<td>$r = 0.007752$</td>
<td>Haas et al., 1999</td>
</tr>
<tr>
<td>Echovirus 12</td>
<td>Ingestion</td>
<td>Exponential</td>
<td>$r = 0.012771$</td>
<td>Haas et al., 1999</td>
</tr>
<tr>
<td>Echovirus 12</td>
<td>Ingestion</td>
<td>Beta-Poisson</td>
<td>$a = 0.374$, $\beta = 186.69$</td>
<td>Regli et al., 1991</td>
</tr>
<tr>
<td>Human adenovirus 4</td>
<td>Inhalation</td>
<td>Exponential</td>
<td>$r = 0.4172$</td>
<td>Haas et al., 1999</td>
</tr>
<tr>
<td>Human caliciviruses</td>
<td>Ingestion</td>
<td>Beta-Poisson</td>
<td>$a = 0.126-0.5$, $\beta = 0.21-0.84$</td>
<td>Soller et al., 2004</td>
</tr>
<tr>
<td>Noroviruses</td>
<td>Ingestion</td>
<td>Exponential</td>
<td>$r = 0.069$</td>
<td>Masago et al., 2006</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Ingestion</td>
<td>Beta-Poisson</td>
<td>$a = 0.253$, $\beta = 0.422$</td>
<td>Regli et al., 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Haas et al., 1999</td>
</tr>
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<td></td>
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<td>Ward et al., 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Teunis et al., 2008</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>Ingestion</td>
<td>Exponential</td>
<td>$r = 0.548576$</td>
<td>Haas et al., 1999</td>
</tr>
</tbody>
</table>
Figure 1-1. Sources of viruses in the environment
Figure 1-2. Summary of virus elution and detection methods
Figure 1-3. Adenovirus removal in full-scale wastewater treatment plants.

Figure 1-4. Enterovirus removal in full-scale waste water treatment plants
Figure 1-5. Norovirus I removal in full-scale waste water treatment plants

Figure 1-6. Norovirus II removal in full-scale waste water treatment plants

Figure 1-7. Virus removal as a function of membrane pore size in bench and pilot scale MBR systems
REFERENCES
REFERENCES


surveillance summaries, 42, 1-22.


Zerda, K. S. (1982). Dissertation, Baylor College of Medicine, Huston, TX.


CHAPTER 2
LITERATURE REVIEW: MEMBRANE BIOREACTORS FOR WATER REUSE IN THE UNITED STATES

Abstract

Water scarcity is a global problem, and the production of wastewater is growing correspondingly along with the ever increasing water consumption. Wastewater can be used as an alternative water resource. Technological developments in treating municipal wastewater, such as membrane bioreactors, provide high quality effluents appropriate for water reuse. In this chapter, we review water reuse issues and standards in the U.S., features and challenges of membrane bioreactor systems, and status of MBR applications in the U.S. It can be concluded that MBR is a superior wastewater treatment technology comparing to conventional activated sludge systems, and it can fulfill the growing water reuse demand.

Keywords: membrane bioreactor, wastewater reuse, pollutant removal, membrane fouling
2.1. Water Reuse in the United States

Generally, the United States is not considered as a country with severe water scarcity (IWMI 2000). However, it has been reported that precipitation is not able to satisfy the withdrawals of fresh water in many regions across the United States, especially in the areas with fast-growing population (Hightower and Pierce 2008). The value of reclaimed water, as an alternative to fresh water sources, has been recognized in many countries. China, Mexico and the U.S. are the top three countries regarding to total volume of reused water, but in China and Mexico, around half of the reused water is untreated wastewater. The U.S. ranks the first for reuse of treated water, and the volume is approximately four times higher than in Saudi Arabia, who takes the second place (Jiménez and Asano 2008). Approximate 9.84 million cubic meters of water is reused/reclaimed per day in the U.S., but that only accounts to 7.4% of the total volume of wastewater generated (Miller et al. 2006, USEPA 2012). The volume of reused water is increasing at an annual rate of 15% in the U.S. (Miller et al. 2006). In the U.S., reclaimed water may serve for many purposes, including urban reuse, industrial reuse, agriculture reuse, environmental reuse, ground water recharge, and potable reuse (USEPA 2012). Agricultural reuse takes the largest portion of 29% of reclaimed water across the country, while landscape/golf course irrigation and recreational impoundment occupy a total of 25% (Bryk et al. 2011; USEPA 2012). The remaining categories of reuses include commercial & industrial reuse, groundwater recharge, geothermal/energy production, natural system restoration, discharge to wetlands and wildlife habitat.

California is the most populous state in the United States, and it has the largest surface and ground water withdrawals. The report of California Recycled Water Policy states that “California is facing an unprecedented water crisis” (State Water Resources Control Board 2013). The history of water reuse in California can be traced back to 1890s. In 2009, the recycled water in California has reached 0.8 km$^3$, but it is still only a small portion when
comparing to the state annual water use, 53 km$^3$ (Water Reuse Association 2009a). According to the California State Water Resources Control Board (2012), agricultural irrigation takes the largest portion (37%) of the reclaimed water. The percentages for landscape irrigation and golf course irrigation are 17% and 7%, respectively. Aquifer recharge, as an indirect potable reuse, has been implemented in California since 1960s (Water Reuse Association 2010), and now its share is 12%. National Water Research Institute (2012) proposed the possibility of direct water reuse in southern California, but it has not been applied so far. It has been estimated that the annual water reuse could reach 2.5 km$^3$ by 2020, and 3.7 km$^3$ by 2030 (California State Water Resources Control Board 2009).

Florida is a leading state in water reuse, where 49% of treated water is reused (Florida Department of Environmental Protection 2012). The total amount of reused water increased from 0.285 km$^3$ in 1986 to 1 km$^3$ in 2012, and the per capita reuse flow is 0.14 million m$^3$ per day in average (Florida Department of Environmental Protection 2013). A percentage of 55% of reclaimed water is used in public access areas, such as parks and schools. 10% of reclaimed water is used to irrigate more than 56.9 k m$^2$ of farmland. Industrial reuse and groundwater recharge take 17% and 13% of reclaimed water, respectively. The rapid growing population has been suggested as the major driving force for the high-level of water reuse (Asano et al. 2007). Economic merits may be another driving force. A total of 74 water reuse utilities in Florida claimed that they provided reclaimed water to their customers for free (Florida Department of Environmental Protection 2013).

As the public is a major stakeholder involved in the decision-making of water management (National Academy of Sciences 2012), social factors play a key role in water reuse (Bouwer 2000). Water reuse projects may fail due to social resistance, even though the treated water can meet certain standards. For example, several indirect potable water reuse projects in the U.S. were strongly opposed by the public. Also, notions like “Toilet to tap” made people uncomfortable, and the social acceptance for water reuse was fairly low. Social
awareness of water reuse is rising in the U.S. A survey conducted by the Water Reuse Research Foundation indicated that people in cities where water reuse projects had been applied were aware of reclaimed water (Water Reuse Association 2009b). However, the levels of water reuse across the U.S. are quite diverse, and it appears that public trust on agencies and confidence on the ability of technologies in pollutant removal were declining (Bruvold 1998). To conclude, water reuse may still be a controversial topic in the U.S. public.

2.2. Water Reuse Standards

Water reuse generally refers to “the use of treated wastewater (reclaimed water) for a beneficial purpose” (NRC 2012). It has been considered as an alternative water source in addition to natural water sources. Water reuse can be classified to direct reuse and indirect reuse. Applications of reclaimed water coming out from treatment facilities directly to target fields, such as agricultural or landscape irrigation, is referred as direct reuse. Indirect reuse, on the other hand, is the discharge of treated water to water bodies (e.g. streams, groundwater aquifer) or storage in a reservoir (e.g. impoundment) before reuse (Levine and Asano 2004). Water reuse can also be categorized into direct potable reuse, indirect potable reuse, and non-potable reuse, in terms of drinking water supply. Non-potable reuse, like agricultural irrigation, has been widely accepted by scientific communities and the general public, whereas potable reuse is still far to reach a consensus (Bouwer 2000; Hartley 2006).

In 1989, the World Health Organization (WHO) published a health guideline for the use of wastewater in agriculture and aquaculture. The water quality standards are mainly focused on microbial pathogens (WHO 1989). After 3 years, Food and Agriculture Organization (FAO) released its guideline for wastewater treatment and use in agriculture and recommended standards for pH, fecal coliforms, and trace elements (Pescod 1992). A lot of countries, to name a few, Germany, Japan, China, and Australia, have established their own
standards for water reuse (Li et al. 2009). The latest water reuse guideline in the United States was published by EPA in 2012. Based on different reuse applications, water quality criteria are set, and the key parameters include pH, biochemical oxygen demand (BOD), total organic carbon (TOC), turbidity or total suspended solids, TSS, fecal coliform, and Cl\textsubscript{2} residual, as shown in Table 2-1. Nitrogen and phosphorus are not included in the EPA water reuse criteria, but they are considered as water quality monitoring parameters, of which the treatment goals in reclaimed water are 1-30 mg/L and 1-20 mg/L, respectively (Levine and Asano 2004). State and local authorities may have additional and stricter standards, depending on the types of reuses. For example, California includes total nitrogen (10mg/L) for indirect potable reuse. North Carolina requires that the level of both Clostridium and coliphage should not exceed 5/100mL (monthly mean) and 25/100mL (daily maximum) in agricultural reuse water (USEPA 2012).

### 2.3. Membrane Bioreactors Technology for Water Reuse

Membrane bioreactors, a combination of activated sludge process with biomass separation by membrane filtration, have become a state-of-the-art technology for municipal and industrial wastewater treatment. Generally there are two ways of integrating the membrane modules into activated sludge process (Cornel and Krause 2008): (1) the submerged configuration, in which the membranes are immersed in the mixed liquor, and permeate is pumped mechanically or by gravity flow; and (2) the side-stream configuration, in which the activated sludge is pumped through membrane module and then recycled, in order to maintain a constant sludge concentration. Comparatively, submerged (immersed) MBR systems are more cost effective and less energy consuming than tubular side-stream systems (Judd 2011; Daigger 2003). Three membrane modules are available for MBRs: hollow fiber, flat sheet and tubular, of which hollow fiber and flat sheet are more prevalent.
(Wachinski 2013). Compared to traditional activated sludge reactors, advantages of MBR include smaller footprint and better effluent quality. Additionally, operation of MBR systems is easier since the performance variability is less, and it significantly reduces the overall area of treatment plant (Choi et al. 2002). MBRs have become a particularly attractive treatment choice for water reuse. In fact, the global MBR market is expanding rapidly.

Membrane bioreactors have been considered as a feasible and promising tool for water reuse (Bixio et al. 2006; Melin et al. 2006; Li et al. 2009; Zanetti et al. 2011). Atasoy et al. (2007) suggested that MBRs can not only reclaim the grey water, but also support the reuse of black water, which is more difficult to be recycled due to its high contamination level. MBR technology is able to treat industrial wastewater and match the requirements for water reuse (Galil and Levinsky 2007; Marrot et al. 2004) as well. Cicek (2003) suggested that MBR technology is capable to remove agricultural wastes, such as pesticides, nitrates and endocrine disrupting compounds, and therefore it can be applied for agricultural wastewater treatment. Cote et al. (2005) described ultrafiltration as “the best available technology” for water reuse, and MBR is a technological option where ultrafiltration can be applied. The fast descending cost of MBR facilities (Bolzonella et al. 2010) make it further more competitive. Howell et al. (2004) concluded four incentives that promote MBR applications for waste treatment: (1) MBR plants are more compact; (2) expansion of plant capacity is simple; (3) the effluent quality is high; (4) the value of reusing is widely recognized.

2.4. Membrane Fouling: Major Challenge of MBR Application

When membrane filtration is carried out in activated sludge, biosolids, colloidal species, and macromolecular species will deposit and accumulate on membrane surface and lead to a flux and permeability decline. This process is called membrane fouling, and it has been considered as the major obstacle and challenge of the development and application of MBRs,
as it increases the maintenance and operational costs (Bouhabila et al. 2001; Cornel and Krause 2008). Ji et al. (2008) published a scanning electron microscope (SEM) photograph of fouled membrane surface, which indicates that bio-film consists of two layers: an inner gel layer and an outer cake layer. The gel layer is thin and compact, and it is strongly attached on the membrane surface. Shin and Kang (2002) suggested that the formation of gel layer is caused by membrane pore blocking and biomass colonization. In contrast, cake layer is thick, porous, and highly compressible (Murase et al. 1995) and it has been suggested that the formation of the cake layer is mainly due to the floc deposition (Hwang and Hsueh 2003). Trans-membrane pressure (TMP) is widely used to indicate the extent of membrane fouling (e.g., Ognier et al. 2002; Ognier et al. 2004; Lee et al. 2001). Higher TMP generally means severe membrane fouling. Membrane fouling could be reversible or irreversible. Reversible fouling is defined as fouling on the membrane surface that can be removed by physical washing, while irreversible fouling, on the other hand, refers to internal fouling into the membrane pores, which can only be removed by chemical clean (Chang et al. 2002). Fouling control is one of the most important issues in MBR operation.

Mixed liquor suspended solids (MLSS) can largely affect the membrane filtration performance (Lee et al. 2001). High MLSS concentrations can accelerate membrane fouling due to large amounts of foulant and rapid deposition of sludge particles on the membrane surface (Sato and Ishii 1991; Han et al. 2005), and it has a direct impact upon cake layer formation (Chang and Kim 2005). However, Hong et al. (2002) observed MLSS exhibited very little influence on permeate flux for the range of 3600-8400 mg/L, and they suggested fouling was independent of MLSS concentration until a very high value was reached. Additionally, Li et al. (2008) even reported a negative correlation between MLSS and membrane fouling resistance.

Extracellular polymeric substances (EPS) in activated sludge are composed of multiple classes of macromolecules such as carbohydrates, proteins, nucleic acids, phospholipids and
other polymeric compounds found at or outside the cell surface and in the intercellular space of microbial aggregates (Judd 2008). High concentration of EPS could affect membrane fouling by increasing viscosity of the mixed liquor (Nagaoka et al. 1996), and filamentous bacteria growth (Meng et al. 2007). The components of soluble microbial products (SMP) include humic and fulvic acids, polysaccharide, proteins, nucleic acid, organic acids, amino acids, antibiotics, steroids, enzymes, structural components of cells, and products of energy metabolism (Rittmann et al. 1987). Carbohydrate component of the SMP were found to be negatively correlated with membrane permeability (Reid et al. 2006).

A positive correlation between food to microorganisms (F/M) ratio and membrane fouling has been found in previous studies. No evidence indicated that F/M ratio had direct impact on membrane fouling, but it could increase the EPS concentrations (Janga et al. 2007), and in turn cause membrane fouling. Additionally, low F/M ratio equals little substrate per unit biomass, which leads to competition among the microorganisms and results in reduction of the net sludge production (Rosenberger et al. 2002). At steady state, low net sludge production leads to higher solids retention time (SRT), and less membrane fouling.

Positive correlations have been found between the presence of filamentous bacteria and membrane fouling. Choi et al. (2002) observed the membrane fouling was most serious under filamentous sludge bulking conditions, in which, filamentous bacteria were predominant in the sludge floc. Three mechanisms that filamentous bacteria may affect the membrane fouling are proposed: (1) Filamentous bacteria could change the floc morphology (Li et al. 2008) and lead to irregular shape of bulking sludge (Meng et al. 2006a); (2) The overgrowth of filamentous bacteria in sludge suspension could form a thick and non-porous cake layer and cause severe membrane fouling (Meng et al. 2006b); (3) Excessive growth of filamentous bacteria could indirectly cause membrane fouling by significantly increasing the extracellular polymeric substances (EPS) concentration and sludge viscosity (Meng et al. 2007).

Hydraulic retention time (HRT) indicates the average time that wastewater stays in
activated sludge reactor. It has been suggested that HRT only has an indirect effect on membrane fouling (Visvanathan et al. 1997; Chang et al. 2002) by affecting other factors, such as MLSS. SRT indicates the average time that suspended solids stay in the activated sludge reactor. SRT is suggested as one of the critical factors controlling SMP concentration in reactor (Lee et al. 2003). With prolonged SRT, concentrations of suspended solids and volatile suspended solids in the bioreactor increase (Huang et al. 2001), and membrane fouling tends to increase due to severer deposition on membrane surface (Han et al. 2005). Nevertheless, similar to HRT, SRT can only indirectly influence membrane fouling (Chang et al. 2002), but the effect of changes in SRT on fouling potential is more sensitive than that of HRT (Jang et al. 2006).

Membrane backwash and chemical clean are the two major ways to mitigate membrane fouling in MBR systems. Membrane backwash is a physical process that removes the loosely attached cake layer. Membrane permeate is commonly used for backwash. The backwash duration varies from seconds to minutes. Chemical clean, on the other hand, is a process that can remove most of the fouling substances from the membrane, and recover the membrane permeability to a large extent.

2.5. Pollutant Removal in MBR Systems

In order to achieve high removal of pollutants, such as nitrogen and phosphate reduction, MBR systems usually consist of multiple stages, as shown in Table 2-2. For example, the wastewater treatment plant (WWTP) in Traverse City, MI is equipped with MBR, and it has a total of six stages: one anaerobic stage, one anoxic stage, three aerobic stages, and one aerobic/membrane stage (Crawford et al. 2006). The operational parameters (MLSS, SRT, recycle ratio, etc.) may be different among stages. Anaerobic treatment is used at the front end of some MBR systems.
2.5.1. Removal of physical and chemical pollutants in MBRs

In conventional activated sludge system, suspended solids are mainly removed by primary sedimentation. Secondary sedimentation/clarification is also responsible for the removal of suspended solids, mostly mixed liquor suspended solids. As shown in Table 2-2, MBR systems are able to achieve high removal for suspended solids and turbidity, due to small membrane pore sizes. It has been suggested that membranes can act as a near-absolute barrier for suspended solids (Wang et al. 2009; Christian et al. 2010). This allows MBRs to be operated at high MLSS levels (Saddoud et al. 2007; Tazi-Pain et al. 2002), that leads to higher removal for pollutants, such as organic substances.

The major removal mechanisms of organic matters in conventional activated sludge systems are adsorption and biodegradation. These two mechanisms are also applied in MBR systems (Cirja et al. 2008), and it has been reported that MBRs can remove organic matter more efficiently comparing to conventional activated sludge systems (Gonzalez et al. 2007). Huang et al. (2000) reported high removals of organic matter in a submerge MBR. As shown in Table 2-2, full-scale MBRs can achieve high removals (usually > 95%) for organic substances.

Chemical precipitation is a traditional method for phosphate removal. This method is reliable, but costs of chemicals and chemical feed systems may be considerable. An alternative method is “Enhanced Biological Phosphorus Removal (EBPR)”. This process consists of an anaerobic stage, where P is released, and an aerobic stage, where P is uptaken (Crocetti et al. 2002; Oehmen et al. 2005). This process is widely used in wastewater treatment plants with lower costs, but it is less stable than chemical treatment (Oehmen et al. 2007).

As discussed above, MBR systems can be operated at high level of MLSS, and this may enhance the bio-processes, such as nitrification and EBPR. Also, membranes can effectively remove nitrogen and phosphate associated with large particles. Compared to other pollutants
however, removal of nitrogen and phosphate in MBR systems appears to be less stable. Extra attention needs to be paid to the removal of nitrogen and phosphate when designing new MBR systems. Conventional methods and technologies may be employed and integrated to the MBRs. For instance, the SymBio® technology, which promotes the simultaneous nitrification and denitrification (SNdN), is applied in an MBR plant at Delphos (OH, USA) (OVIVO case study 2011).

2.5.2. Removal of pathogens in MBRs

Removing microbial pathogens is critical for water reuse safety. The water reuse guidelines set microbial requirements in terms of fecal coliform. Bacteria removal in full scale MBR systems are summarized in Table 2-3. It can be seen that most full-scale MBRs can achieve high removal efficiency for bacteria, and membrane pore size appears to be an important factor. Aidan et al. (2007) reported that an MBR equipped with 0.8 μm ceramic membrane could only remove 39% coliform bacteria, while high or complete removal was reached by using membranes with smaller pore sizes (Herrera-Robledo et al. 2010; Hirani et al. 2010; Saddoud et al. 2007).

Compared to bacterial indicators, investigations for the removal of specific pathogens in MBRs are relatively rare. Tests for some pathogenic bacteria (e.g. Salmonella spp., Campylobacter spp., Cryptosporidium, etc.) were applied in several MBR studies, but no concentrations in the influent (raw wastewater) were detected (Winward et al. 2008; Jefferson et al. 2004).

It has been reported that more than 100 types of enteric viruses are excreted in human feces and present in contaminated waters (Melnick et al. 1978; Havelaar et al. 1993). Enteric viruses pose a considerable threat to human health due to their low infectious dose and long survival in the environment. Table 2-4 shows the removal of bacterial viruses (coliphages)
and human viruses, such as adenoviruses, enteroviruses and noroviruses in full-scale MBRs. Bench-scale MBR with hydrophobic polyvinylidene fluoride membrane (pore size = 0.22 µm) could remove 99% of poliovirus, while ultrafiltration could achieve a complete rejection due to smaller pore size (Madaeni et al. 1995). Presence of biomass, low trans-membrane pressure and stirring could enhance the removal (Madaeni et al. 1995). MBR systems with higher HRT and lower SRT seemed to be more efficient in removing viruses (Wu et al. 2010).

Gallas-Lindemann et al. (2013) reported high removal efficiencies for *Giardia cyst* (99.4%) and *Cryptosporidium* (94.2%) in a full-scale MBR. Herrera-Robledo et al. (2010, 2011) reported high removal of helminth eggs in a bench-scale anaerobic MBR with ultrafiltration membranes. By using a pilot-scale anaerobic MBR, Saddoud et al. (2006, 2007b, 2009) observed complete removal for helminth ova and protozoan cysts. Abdel-Shafy (2008) investigated the removal of protozoan cysts, helminthes eggs, nematodes in a pilot submerged MBR, and the results indicated that the MBR was able to reject all of these microorganisms.

MBR treatment is usually followed by disinfection. Three traditional methods are available for disinfection, namely, chlorine disinfection, ozone disinfection, and UV disinfection. Chlorine disinfection is the most commonly used method in conventional activated sludge plants. It is cost-effective and well-established, but the residual and forms of chlorine could be toxic, and further dechlorination may be required (EPA 1991a). Ozone is more effective than chlorine for disinfection, without any residual left in effluent, however, it is very reactive and corrosive, and costs for the method could be considerable (EPA 1991b). UV disinfection leaves no residual in the effluent, but high water turbidity may cause it to be less effective (EPA 1991c). As described above, high TSS removal can be achieved in MBRs, which makes UV become a feasible and preferable disinfection process in MBR plants. In fact many MBR plants in the United States use UV for disinfection, to name a few: Duvall WWTP (WA), Nantucket WWTP (MA), Cauley Creek WWTP (GA).
2.6. Comparison between Conventional Activated Sludge (CAS) System and MBR

Similar to conventional treatment, pretreatment to remove large objects and separate solids and grease from wastewater, is required before the raw wastewater enters MBR systems. Typical components of pretreatment include coarse screen, grit, grease trap, fine screen, equalization, and primary sedimentation. Activated sludge is a component in both CAS and MBR, but different microbial community structures have been observed between the two systems in many previous studies (Gao et al. 2004; Ouyang and Liu 2009; Li et al. 2004; Silva et al. 2010; Munz et al. 2008). Furthermore, sludge floc size in MBR systems is smaller comparing to CAS systems (Cicek et al. 1999; Holbrook et al. 2005), which implies higher oxygen transfer rate (Liu et al. 2001).

It has been widely accepted that in general MBRs have superior and stable performance in pollutant removal comparing to conventional activated sludge. Soriano et al. (2003) obtained higher carbon and nitrogen removal in an MBR system. Munz et al. (2008) attributed more efficient COD removal and nitrification process in MBR to different microbial community compositions and distributions. Cirja et al. (2008) concluded that sorption and biodegradation were the major mechanisms of organic micropollutants removal in both CAS and MBR. Although no substantial difference was found between these two systems, the potential capability of MBR for high organic load was suggested. Gonzalez et al. (2007) showed that concentrations of COD, NH$_4^+$ and total suspended solids (TSS) in MBR effluent were consistently lower than CAS, and it was independent from the influent concentrations. Bernhard et al. (2006) suggested MBR provided better removals for non-adsorbing persistent polar pollutants, such as sulfophenylcarboxylates. Holbrook et al. (2005) concluded that accumulation of nondegradable chemical oxygen demand in MBR was responsible for smaller average floc size and higher observed biological yield coefficient comparing to CAS. Wei et al. (2003) reported that worm growth was much faster in CAS reactor, which might affect effluent quality. Pauwels et al. (2006) found that MBR offered
similar removal for ammonium nitrogen and ethinylestradiol when treating hospital water, but had better performance in rejecting indicator microorganisms, such as fecal coliforms. Simmons and Xagoraraki (2011) found higher reduction of human adenoviruses and enteroviruses in an MBR plant as compared to CAS plants.

2.7. Applications of MBR with Water Reuse in the U.S.

So far, more than 6000 MBR plants have been installed worldwide, and over 600 of them are in the United States (Kafka 2013). Table 2-5 shows MBR plants in the U.S. with water reuse applications. Many MBR facilities provide no information in sight of water reuse, where it is more likely that water reuse is not applied.

As shown in Table 2-5, most MBR plants in the United States began their service after 2004. In fact, a lot of these plants served as conventional WWTPs for decades and were upgraded to MBFRs in the 21st Century. For example, the Union Rome WWTP was initially built in 1986, and commissioned as an MBR in 2009. The maximum capacities of most MBR plants are below 38,000 m$^3$/d (10 mgd), but they could reach 95,000 m$^3$/d (25 mgd). Construction of an MBR typically takes 1 - 3 years, depending on the size. Additionally, the capacities of MBFRs are usually expandable. Kubota and GE appear to be the most prevalent membrane suppliers for MBR facilities across the United States, and they are also the major membrane suppliers in Europe (Melin et al. 2006). GE is known for its ZeeWeed membranes, which are a type of ultrafiltration hollow fiber membrane, while Kubota generally provides flat-plate microfiltration membranes.

Reclaimed water from MBR systems with reuse programs in the U.S. is mostly used for non-potable purposes, among which land irrigation appears to be one of the most common applications (e.g. Upper Sweetwater WWTP, GA; Corona WWTP, CA). Other non-potable reuse applications include industrial reuse (e.g. Redlands WWTP, CA) and fire protection (e.g.
Red Hawk Casino WWTP, CA). Groundwater recharge has been considered as a sustainable and economical way of water storage without eco-environmental problems (Bouwer et al., 2000). Some MBR plants in the U.S. have injected their treated water to underground aquifers, as a type of indirect potable reuse (e.g. Shelton WWTP, WA; Upper Wallkill WWTP, NJ). Although direct potable reuse has been proposed by Water Reuse Association (2009a, 2010), no evidence shows that treated water from MBRs is to be applied for such purpose in the United States. It is notable that many MBR WWTPs discharged their effluent directly to river without any reuse (e.g. Crooked Creek WRF, GA; Nantucket WWTP, MA), even though the water quality meets the standard for water reuse. In addition, the wastewater treatment facilities with water reuse applications may also discharge a portion of their reclaimed water, depending on the demands. For example, the demand of reclaimed water for agricultural irrigation may be low in winter, while treated wastewater is produced all year around.

2.8. Conclusions

Due to the ever-growing water demand and fierce water crisis all over the world, water reuse and water reclamation, as alternatives to natural water resources, are drawing more and more attention. In the United States, the levels of water reuse are low in general, but increasing fast. Agricultural irrigation is generally the most common application of reused water across the country; other reuses include land irrigation, aquifer recharge, commercial & industrial reuse, wetlands and wildlife habitat. The U.S. Environmental Protection Agency (USEPA) has established guidelines and criteria for water reuse in 2012.

Membrane bioreactor (MBR) technology has been proven as an effective method in wastewater treatment, and provides effluent that meets EPA water reuse criteria. In the U.S., a number of conventional WWTPs have been upgraded to MBR plants, and effluent water is being reused. Previous studies have demonstrated that MBRs have smaller footprints, less
land occupancy, and higher removal efficiencies of pollutants, especially organic micropollutants and emerging pathogens in contrast to conventional activated sludge systems. It has been shown that removal of bacterial indicators and pathogenic viruses in MBR systems as compared to CAS. Membrane fouling is considered as the main obstacle in MBRs, and fouling control is one of the key issues in MBR operation. In the U.S., treated water from MBR plants is more likely to be reused for land irrigation, such as lawns and golf courses. Although membrane technology has been studied for decades and MBR facilities are widely installed in the U.S., the reuse level of reclaimed water from MBRs appears to be low. However, great potential of water reuse is expected when social, economical and environmental drivers are activated.
Table 2-1. Water quality criteria of EPA guideline for water reuse (EPA 2012)

<table>
<thead>
<tr>
<th>Reuse Category</th>
<th>Non-potable reuse I</th>
<th>Non-potable reuse II</th>
<th>Indirect Potable Reuse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban reuse (restricted), Processed food corps, Non-food corps, Impoundments (restricted), Environmental reuse, Industrial reuse</td>
<td>Urban reuse (unrestricted), Impoundments (unrestricted), Food corps</td>
<td>Groundwater recharge into potable aquifers, Augmentation of surface water supply reservoirs</td>
</tr>
<tr>
<td>pH</td>
<td>6.0 – 9.0</td>
<td>6.0 – 9.0</td>
<td>6.5 – 8.5</td>
</tr>
<tr>
<td>Organic matter</td>
<td>≤ 30 mg/L BOD</td>
<td>≤ 10 mg/L BOD</td>
<td>≤ 2 mg/L TOC of wastewater origin</td>
</tr>
<tr>
<td>Turbidity or TSS</td>
<td>≤ 30 mg/L TSS</td>
<td>≤ 2 NTU</td>
<td>≤ 2 NTU</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>≤ 200 fecal coliform /100 mL</td>
<td>No detectable fecal coliform /100 mL</td>
<td>No detectable fecal coliform /100 mL</td>
</tr>
<tr>
<td>Cl₂ residual</td>
<td>1 mg/L Cl₂ (min.)</td>
<td>1 mg/L Cl₂ (min.)</td>
<td>1 mg/L Cl₂ (min.)</td>
</tr>
</tbody>
</table>
Table 2-2. Pollutant removal in selected full-scale MBR plants in the U.S.

<table>
<thead>
<tr>
<th>Plant Configuration and MBR Module</th>
<th>Pollutant Removal (Influent/Effluent)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leoni Twp WWTP, MI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic + pre-aeration + membrane tanks</td>
<td>TSS  ~170/2</td>
<td>Kafka 2013</td>
</tr>
<tr>
<td>Kubota® immersed flat sheet membrane</td>
<td>BOD ~170/2</td>
<td></td>
</tr>
<tr>
<td>UV disinfection</td>
<td>NH₃-N 23/0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP  5/0.24</td>
<td></td>
</tr>
<tr>
<td><strong>The Hamptons WWTP, GA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic + aerobic stages</td>
<td>TSS  200/2</td>
<td>Enviroquip case study 2012</td>
</tr>
<tr>
<td>Kubota® flat sheet membrane</td>
<td>BOD  200/3</td>
<td></td>
</tr>
<tr>
<td>Chemical disinfection</td>
<td>TN  40/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP  10/0.13</td>
<td></td>
</tr>
<tr>
<td><strong>Ken’s Foods WWTP, MA (food industry)</strong></td>
<td>TSS  12,000/2</td>
<td>Judd 2011</td>
</tr>
<tr>
<td>Anaerobic Kubota systems</td>
<td>BOD  18,000/16</td>
<td></td>
</tr>
<tr>
<td>Kubota® immersed flat sheet membrane</td>
<td>COD 34,000/200</td>
<td>Judd 2011</td>
</tr>
<tr>
<td>No disinfection unit reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Traverse City WWTP, MI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic + anoxic + aerobic stages + membrane tanks</td>
<td>TSS  248/1</td>
<td>USEPA 2007; Judd 2011</td>
</tr>
<tr>
<td>ZeeWeed® immersed hollow fiber membrane</td>
<td>BOD  280/2</td>
<td></td>
</tr>
<tr>
<td>UV disinfection</td>
<td>NH₃-N 27.9/0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP  6.9/0.7</td>
<td></td>
</tr>
<tr>
<td><strong>Cauley Creek WRF, GA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic + swing zone + 2 aerobic stages + membrane tanks</td>
<td>TSS  174/3.2</td>
<td>USEPA 2007; Badran 2004</td>
</tr>
<tr>
<td>ZeeWeed® immersed hollow fiber membrane</td>
<td>BOD  182/2</td>
<td></td>
</tr>
<tr>
<td>UV disinfection</td>
<td>COD  398/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TKN  33/1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH₃-N 24.8/0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP  5/0.1</td>
<td></td>
</tr>
<tr>
<td><strong>Calls Creek WWTP, GA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic + aerobic + membrane tanks</td>
<td>TSS  248/1</td>
<td>USEPA 2007; Pellegrin and Hatcher 2008</td>
</tr>
<tr>
<td>Siemens/U.S. Filter Systems Orbal® system</td>
<td>Turbidity  NA/0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOD  145/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH₃-N 14.8/0.21</td>
<td></td>
</tr>
<tr>
<td>UV disinfection</td>
<td>Turbidity  NA/0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP  0.88/0.28</td>
<td></td>
</tr>
<tr>
<td><strong>Redlands WWTP, CA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic + aerobic + membrane tanks</td>
<td>TSS  130/5</td>
<td>General Electric (GE) case study 2011</td>
</tr>
<tr>
<td></td>
<td>Turbidity  NA/0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOD  160/5</td>
<td></td>
</tr>
<tr>
<td>ZeeWeed® reinforced hollow fiber UF membrane</td>
<td>TN  24/10</td>
<td></td>
</tr>
<tr>
<td>Chlorine disinfection</td>
<td>TN  24/10</td>
<td></td>
</tr>
<tr>
<td><strong>Santa Paula WWTP, CA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic + aerobic + membrane tanks</td>
<td>TSS  210/5</td>
<td>Carollo Engineers 2006</td>
</tr>
<tr>
<td></td>
<td>BOD  320/5</td>
<td></td>
</tr>
<tr>
<td>PURON® membrane filtration modules</td>
<td>TKN  53/7</td>
<td></td>
</tr>
<tr>
<td>UV disinfection</td>
<td>TDS  1300/1000</td>
<td></td>
</tr>
</tbody>
</table>

*Concentrations of some pollutants in the influent are extrapolated based on the concentrations in the effluent and removal efficiencies. Units for turbidity are NTU, and for other parameters are mg/L.
Table 2-3. Bacteria removal in full-scale MBRs

<table>
<thead>
<tr>
<th>MBR Type (membrane pore size)</th>
<th>Bacterial Indicators</th>
<th>Removal Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submerged hollow fiber MBR (0.035 μm)</td>
<td>Total coliform</td>
<td>Before disinfection: NA</td>
<td>Bassyouni et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Total viable count</td>
<td>After disinfection: Up to 4 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>3.6 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total coliform</td>
<td>4.7 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>4.1 logs</td>
<td></td>
</tr>
<tr>
<td>Submerged hollow sheet (0.2 μm)</td>
<td>Total coliform</td>
<td>3.6 logs</td>
<td></td>
</tr>
<tr>
<td>Submerged MBR (0.4 μm)</td>
<td><em>E. coli</em></td>
<td>4.6 logs</td>
<td></td>
</tr>
<tr>
<td>Submerged flat sheet MBR (0.4 μm)</td>
<td><em>E. coli</em></td>
<td>Complete removal</td>
<td></td>
</tr>
<tr>
<td>Parallel-panel submerged MBR (0.45 μm)</td>
<td><em>E. coli</em></td>
<td>Complete removal</td>
<td></td>
</tr>
<tr>
<td>Submerged flat sheet MBR (0.4 μm)</td>
<td>Total coliform</td>
<td>6.02 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Thermo-tolerant</em></td>
<td>6.72 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>coliforms</td>
<td>7.32 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fecal coliforms</em></td>
<td>6.98 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>Complete removal</td>
<td></td>
</tr>
<tr>
<td>Microfiltration MBR (&lt;0.4 μm)</td>
<td><em>Enterococci</em></td>
<td>6.77 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>Complete removal</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Enterococci</em></td>
<td>5.77 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fecal coliforms</em></td>
<td>5.37 - &gt; 6.85 logs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Enterococci</em></td>
<td>Not enhanced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.82 - 7.49 logs</td>
<td>Not enhanced by &gt; 0.30 log</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fecal coliforms</em></td>
<td>5.34 - 7.23 logs</td>
<td></td>
</tr>
</tbody>
</table>

Reference:
- Bassyouni et al. 2006
- Guerra 2010
- Wen et al. 2004
- De Luca et al. 2013
- Sima et al. 2011
- Zanetti et al. 2010
- Francy et al. 2012
### Table 2-4. Virus removal in full-scale MBRs

<table>
<thead>
<tr>
<th>MBR Type (membrane pore size)</th>
<th>Virus</th>
<th>Removal Efficiency Before disinfection</th>
<th>Removal Efficiency After disinfection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submerged flat sheet MBR (0.4 μm)</td>
<td>F-specific coliphage</td>
<td>5.82 logs</td>
<td>Complete removal</td>
<td>Zanetti et al. 2010</td>
</tr>
<tr>
<td></td>
<td>Somatic coliphage</td>
<td>4.44 logs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteriophages infecting <em>bacteroides fragilis</em></td>
<td>Complete removal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submerged hollow fiber MBR (0.1 μm)</td>
<td>HAdV</td>
<td>4.1 - 5.6 logs</td>
<td>NA</td>
<td>Kuo et al. 2010</td>
</tr>
<tr>
<td>Submerged MBR (0.4 μm)</td>
<td>Norovirus I</td>
<td>NA</td>
<td>0 - 5.3 logs*</td>
<td>da Silva et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Norovirus II</td>
<td>NA</td>
<td>0 - 5.5 logs*</td>
<td></td>
</tr>
<tr>
<td>Submerged hollow fiber MBR (0.1 μm)</td>
<td>HAdV</td>
<td>3.4 - 4.5 logs*</td>
<td>Removal enhanced by ~0.8 log*</td>
<td>Simmons and Xagoraraki 2011</td>
</tr>
<tr>
<td></td>
<td>Enterovirus</td>
<td>2.9 - 4.6 logs*</td>
<td>Removal enhanced by ~0.4 log*</td>
<td></td>
</tr>
<tr>
<td>Submerged hollow fiber MBR (0.1 μm)</td>
<td>HAdV</td>
<td>4.1 - 6.3 logs</td>
<td>NA</td>
<td>Simmons et al. 2011</td>
</tr>
<tr>
<td></td>
<td>Enterovirus</td>
<td>4.1 - 6.8 logs</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norovirus (II)</td>
<td>3.5 - 4.8 logs</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F-specific coliphage</td>
<td>&gt;4.58 - 6.6 logs</td>
<td>Not enhanced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Somatic coliphage</td>
<td>2.67 – 4.04 logs</td>
<td>Removal enhanced by &gt;2.18 logs^</td>
<td>Francy et al. 2012</td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
<td>2.38 - &gt;4.86 logs</td>
<td>Not enhanced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterovirus</td>
<td>&gt;2.2 – 4.74 logs</td>
<td>Not enhanced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norovirus I</td>
<td>&gt;1.51 – 3.32 logs</td>
<td>Not enhanced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Culturable viruses</td>
<td>&gt;1.99 - &gt;3.61 logs</td>
<td>Not enhanced</td>
<td></td>
</tr>
<tr>
<td>Flat sheet submerged MBR (0.4 μm)</td>
<td>Somatic coliphage</td>
<td>4.43 – 4.44 logs</td>
<td>NA</td>
<td>Luca et al. 2013</td>
</tr>
<tr>
<td>Parallel-panel submerged MBR (0.45 μm)</td>
<td>F-specific coliphage</td>
<td>5.81 – 5.83 logs</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norovirus</td>
<td>0.9 – 6.8 logs</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sapovirus</td>
<td>1.7 – 4.1 logs</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*Read from graphs.
^Median value
Table 2-5. Selected MBR wastewater treatment facilities in the U.S. with water reuse*

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Commission Year</th>
<th>Peak Capacity</th>
<th>Membrane manufacturer</th>
<th>Water Reuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnation WWTP</td>
<td>King County, WA</td>
<td>2008</td>
<td>1817 m³/d</td>
<td>GE</td>
<td>Irrigation</td>
</tr>
<tr>
<td>Brightwater WWTP</td>
<td>King County, WA</td>
<td>2011</td>
<td>117,348 m³/d</td>
<td>ZeeWeed (GE)</td>
<td>Irrigation, industrial reuse</td>
</tr>
<tr>
<td>Cauley Creek WRF</td>
<td>Fulton County, GA</td>
<td>2004</td>
<td>18,927 m³/d</td>
<td>ZeeWeed (GE)</td>
<td>Land irrigation, lawn watering, discharge</td>
</tr>
<tr>
<td>Fowler WRF</td>
<td>Forsyth County, GA</td>
<td>2004</td>
<td>9,464 m³/d</td>
<td>Zenon (GE)</td>
<td>Land irrigation</td>
</tr>
<tr>
<td>Spokane County WRF</td>
<td>Spokane County, WA</td>
<td>2011</td>
<td>30,283 m³/d</td>
<td>GE</td>
<td>Industrial, urban irrigation, wetlands restoration, aquifer recharge</td>
</tr>
<tr>
<td>Yellow River WRF</td>
<td>Gwinnett County, GA</td>
<td>2012</td>
<td>69,273 m³/d</td>
<td>ZeeWeed (GE)</td>
<td>Non-potable purpose or direct discharge to river</td>
</tr>
<tr>
<td>James Creek WRF</td>
<td>Forsyth County, GA</td>
<td>2006</td>
<td>3,785 m³/d</td>
<td>Enviroquip (Kubota)</td>
<td>Land irrigation</td>
</tr>
<tr>
<td>Johns Creek Environmental Campus</td>
<td>Fulton County, GA</td>
<td>2009</td>
<td>56,781 m³/d</td>
<td>Zenon</td>
<td>Irrigation, toilet water, fire protection</td>
</tr>
<tr>
<td>Pooler WWTP</td>
<td>Chatham County, GA</td>
<td>2004</td>
<td>9,464 m³/d</td>
<td>ZeeWeed</td>
<td>Irrigation to golf course</td>
</tr>
<tr>
<td>Upper Sweetwater WWTP</td>
<td>Paulding County, GA</td>
<td>Before 2009</td>
<td>3,785 m³/d</td>
<td>Kubota</td>
<td>Irrigation to golf course</td>
</tr>
<tr>
<td>Yakama Nation Legends Casino WWTP</td>
<td>Yakima County, WA</td>
<td>2008</td>
<td>1,363 m³/d</td>
<td>Enviroquip (Kubota)</td>
<td>Lawn irrigation, discharge</td>
</tr>
<tr>
<td>Shelton WWTP</td>
<td>Mason County, WA</td>
<td>2012</td>
<td>15,142 m³/d</td>
<td>Ovivo (Kubota)</td>
<td>Regional Plan participants, Ground water recharge.</td>
</tr>
<tr>
<td>Red Hawk Casino WWTP</td>
<td>CA</td>
<td>2008</td>
<td>2,650 m³/d</td>
<td>Kubota</td>
<td>Toilet flushing, fire protection, landscaping</td>
</tr>
<tr>
<td>American Canyon WWTP</td>
<td>Napa County, CA</td>
<td>2002</td>
<td>14,195 m³/d</td>
<td>ZeeWeed</td>
<td>Vineyard and golf course irrigation, discharge</td>
</tr>
<tr>
<td>Corona WWTP</td>
<td>Riverside County, CA</td>
<td>2001</td>
<td>3,785 m³/d</td>
<td>ZeeWeed</td>
<td>Landscape irrigation, discharge</td>
</tr>
<tr>
<td>Marco Island WWTP</td>
<td>Collier County, FL</td>
<td>2007</td>
<td>11,356 m³/d</td>
<td>ZeeWeed</td>
<td>Land irrigation</td>
</tr>
<tr>
<td>Ironhouse Sanitary District WWTP</td>
<td>Contra Costa County, CA</td>
<td>2011</td>
<td>32,555 m³/d</td>
<td>ZeeWeed</td>
<td>Irrigation, discharge</td>
</tr>
</tbody>
</table>
Table 2-5. (Cont’d)

<table>
<thead>
<tr>
<th>Facility Name</th>
<th>County, State</th>
<th>Year</th>
<th>Flow Rate (m³/d)</th>
<th>Membrane Type</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallingwater Conservancy WWTP</td>
<td>Fayette, PA</td>
<td>2003</td>
<td>3,331</td>
<td>ZeeWeed</td>
<td>Flush water, garden irrigation</td>
</tr>
<tr>
<td>Redlands WWTP</td>
<td>San Bernardino, CA</td>
<td>2004</td>
<td>24,984</td>
<td>ZeeWeed</td>
<td>Industrial reuse</td>
</tr>
<tr>
<td>The Hamptons WRF</td>
<td>Forsyth, GA</td>
<td>2003</td>
<td>1041</td>
<td>Kubota</td>
<td>Land irrigation</td>
</tr>
<tr>
<td>Santa Paula WWTP</td>
<td>Ventura, CA</td>
<td>2010</td>
<td>27,255</td>
<td>Koch membrane</td>
<td>Irrigation</td>
</tr>
<tr>
<td>Upper Wallkill WWTP</td>
<td>Sussex, NJ</td>
<td>2010</td>
<td>1,003</td>
<td>Kubota</td>
<td>Groundwater discharge</td>
</tr>
</tbody>
</table>

*Many MBR facilities in the United States are not included in this table due to lack of information regarding water reuse.

MGD = Million Gallons per Day
WWTP = Wastewater Treatment Plant
WRF = Water Reclamation Facility
Discharge = discharge to the environment (rivers, creeks, canals etc.)
REFERENCES
REFERENCES


OVIVO case study, (2011). Delphos, OH, Wastewater Treatment Plant. [http://www.ovivowater.us/content/files/data/Delphos%20Case%20Study_d76b4cbe572a45c194bcb622e262bae5.pdf](http://www.ovivowater.us/content/files/data/Delphos%20Case%20Study_d76b4cbe572a45c194bcb622e262bae5.pdf)


United States Environmental Protection Agency, (2007). Wastewater Management Fact Sheet. [https://www.google.com/#q=Wastewater+Management+Fact+Sheet++1++Membrane+Bioreactors](https://www.google.com/#q=Wastewater+Management+Fact+Sheet++1++Membrane+Bioreactors)


CHAPTER 3

HUMAN ADENOVIRUS REMOVAL BY HOLLOW FIBER MEMBRANES: EFFECT OF MEMBRANE FOULING BY SUSPENDED AND DISSOLVED MATTER

Abstract

Virus removal in membrane bioreactors is of concern since membrane pore size can be larger than the size of certain viruses. In this study, we evaluated removal of human adenovirus 40 (HAdV 40) by hollow fiber ultrafiltration (UF, $d_{\text{pore}} = 0.04 \ \mu m$) and microfiltration (MF1, $d_{\text{pore}} = 0.22 \ \mu m$; MF2, $d_{\text{pore}} = 0.45 \ \mu m$) membranes operated in the constant flux regime and in the presence of aeration. Individual and combined impacts of suspended (SiO$_2$ microspheres) and dissolved (Aldrich humic acid) foulants on permeate flux and virus removal were determined and compared. Average removal of HAdV 40 from DI water by UF, MF1 and MF2 membranes was 2.3 log, 0.7 log and 0.7 log, respectively. The observed decrease in HAdV 40 removal due to SiO$_2$ fouling (δLRV of -1.2 and -0.2 for UF and MF1 respectively) was attributed to the cake-enhanced accumulation of viruses at the membrane surface. In contrast, fouling by humic acid led to higher virus removals (δLRV of 0.8 and 1.2 for UF and MF1, respectively), which was attributed to pore blockage by humic acid. In experiments with MF2 membrane, neither humic acid nor SiO$_2$ had statistically significant effects on HAdV 40 removal. Combined fouling by humic acid and SiO$_2$ led to HAdV 40 removal that appeared to a superposition of individual contributions of these constituents. The results indicate that the extent of fouling is not a reliable predictor of virus removal. Instead, feed water composition and membrane pore size together govern virus removal with fouling mechanisms playing a key mediating role: pore blockage improves virus removal while cake formation can either increase or decrease virus removal depending on cake properties.
Keywords: microfiltration, ultrafiltration, membrane fouling, virus removal, adenovirus
3.1. Introduction

More than 150 types of enteric viruses have been found in contaminated waters (Leclerc et al. 2000; Wong et al. 2012; Havelaar et al. 1993; Melnick et al. 1978). Because of their low infectious dose and long survival in the environment viruses pose a considerable threat to human health. Human adenovirus (HAdV) is one of enteric viruses on the U.S. EPA’s contaminant candidate list. Various species of HAdV can cause a range of diseases (Heim et al. 2003; Jones et al. 2007); for example, HAdV-F is the known etiological agent of gastroenteritis while HAdV-B and HAdV-E may lead to acute respiratory diseases. A double-strand DNA virus, HAdV is one of largest virions ranging from 70 to 140 nm in size (Xagoraraki et al. 2014). What makes HAdV particularly problematic is its resistance to UV disinfection (Ko et al. 2005; Nwachuku et al. 2005; Baxter et al. 2007) with UV dosages as high as 217.1 mW/cm² required for 99.99% deactivation of HAdV 40 (Thurston-Enriquez et al. 2003). The large size of HAdV and its resistance to UV light point to the promise of membrane filters as a treatment process for removing this virus from water.

Although some pathogen removal occurs during wastewater treatment, even advanced technologies may not provide an absolute barrier for viruses. Indeed, recent studies report presence of human enteric viruses in the effluents of state-of-the art treatment facilities such as membrane bioreactors (MBR) plants (Kuo et al. 2010; Simmons et al. 2011) and drinking water treatment plants (Sedmak et al. 2005; Albinana-Gimenez et al. 2009). MBRs can achieve high and stable removal efficiency for chemical oxygen demand (Pankhania et al. 1994; Beaubien et al. 1996), biochemical oxygen demand (Kishino et al. 1996), nitrogen (Kishino et al. 1996; Gujer et al. 1999), phosphorus (Schaum et al. 2005), and coliform bacteria (Van der Roest et al. 2002). Virus removal, however, has not been a criterion in the design and operation of MBR plants. In fact, some MBRs employ membranes with the nominal pore size larger than the size of a typical virus (20 – 200 nm), in which case
membrane fouling and cleaning may control virus removal.

Multiple studies evaluated virus removal as a function of membrane and feed properties; some of this work has employed bacteriophages as human virus surrogates. Langlet et al. showed an increase in MS-2 phage removal with a decrease in the membrane pore sizes (Langlet et al. 2009). Lu et al. (2013) found a strong linear correlation between MS-2 log removal and permeability of ultrafilters in the presence of foulants in the feed: on average, fouling increased MS-2 removal by 1.23 logs. Working with the same type of phage, Jacangelo et al. reported that membrane fouling contributed up to 2.6 logs removal of MS-2, which was much more significant comparing to physical sieving/adsorption (0.3 log) and cake layer formation (0.1 – 0.5 log) (Jacangelo et al. 1995). Wu et al. (2010) reported that gel layer contributed to the removal of somatic coliphage removal, more so at a higher permeate flux. High removals of T4 coliphage have been reported and partly attributed to the formation of a cake layer formed on membrane surface (Lv et al. 2006; Zheng et al. 2005; Zheng and Liu 2007). Shirasaki et al. (2008) carried out filtration experiments in a coagulation–MF system and concluded that irreversible fouling played a more important role than reversible fouling in enhancing virus removal. Farahbakhsh and Smith (2004) investigated coliphage removal from secondary effluent of wastewater treatment plant by microfiltration membrane and reported that fouled membranes rejected viruses more effectively. Composition of the feed water (pH, ionic strength, presence of divalent actions and organic matter) and pretreatment were suggested as key factors governing virus removal (van Voorthuizen et al. 2001; Huang et al. 2012; Fiksdal and Leiknes 2006; Madaeni et al. 1995; Matsushita et al. 2005; Zhu et al. 2005).

To our knowledge, there have been only six studies on adenovirus removal by membranes with all this work performed in the context of MBR treatment. Sedmak et al. reported presence of HAdV in Milwaukee’s Jones Island wastewater treatment plant (WWTP) effluent although in a much smaller fraction of samples and much lower titers than in the
influent (Sedmak et al. 2005). Albinana-Gimenez et al. (2009) reported sporadic qPCR-positive but PFU-negative results in the effluent from drinking water treatment plants. (In contrast, culturable HAdV in MBR effluent was measured in effluents of each of 10 conventional WWTPs sampled by Hewitt et al. (2011)). Kuo et al. (2010) showed that HAdV species A, C, and F were removed only partially in the Traverse City MBR WWTP and showed that with the average HAdV removal of 5.0±0.6 logs over the 8 month long study, the effluent contained on average ~ 10³ HAdV particles/L. In their study of enteric virus removal in conventional WWTPs and microfiltration MBR WWTPs (equipped with Kubota membranes), Francy et al. (2012) showed that HAdV was detected by q-PCR in a subset of MBR effluent samples both before and after UV disinfection. In a survey of virus removal in nine MBR WWTPs employing different kinds of membranes (tubular, hollow fiber and flat sheet; MF and UF), Hirani et al. (2013) reported that adenoviruses were detected in effluents of all MBR facilities sampled; this result was consistent with the findings by Kuo et al. (2010) and was particularly striking because enteroviruses, rotaviruses and hepatitis A viruses were absent in all samples. The authors tentatively attributed this finding to the fact that HAdV concentration in the influent is typically very high and concluded that “additional research and risk assessment on presence of adenovirus in MBR effluents is warranted.” In a follow-up study (Hirani et al. 2014) with four different membrane systems, these authors showed that adenoviruses were always detected in MBR filtrate samples by PCR regardless of whether the membrane was breached (effluent turbidity > 0.5 NTU) or cleaned (0.2% NaClO).

The objective of the present work was to elucidate mechanisms of HAdV removal by membranes in the presence of foulants in the feed. To facilitate mechanistic insights, we employed two well characterized model foulants (humic acid and silica particles), three commercially available hollow fiber membranes (with pore sizes typical for membranes used in MBRs), and filtration conditions that matched, to the extent possible, the protocol used at
full-scale MBR facilities (i.e. constant flux regime, aeration).

3.2. Materials and Methods

3.2.1. Cell culture experiment and virus incubation

A549 cell line has been suggested as an efficient cell line for HAdV (Witt and Bousquet, 1988; Lee et al., 2004), and it was selected in this study. A549 cells (ATCC, cell passage were incubated at 37°C with growth medium (minimum essential medium with 10% fetal bovine serum, L-glutamine, Earle’s salts) until confluence of cell layer reached 90%. Used media was discarded from the flask, and HAdV 40 was added and incubated at 37°C with growth media (2% fetal bovine serum) until cytopathic effect became apparent. In order to isolate viruses from cell debris, virus suspension was centrifuged at 400 g for 4 min, and then filtered through 0.22 μm syringe-driven filter (Millipore). Filtered virus stock suspension had HAdV concentration of approximately 10^{10} copies/mL and was stored at -80°C before use.

3.2.2. Membrane preparation

Three types of hollow fiber membranes were used in this study. The characteristics of the membranes are shown in Table 3-1. The hollow fibers were cut into 80 cm long segments and assembled by looping and potting them in a short (~10 cm) piece of 1/2” ID PTFE tubing using an adhesive (Loctite). Each membrane bundle (12 loops of 0.45 μm and 0.22 μm membranes and 8 loops of 0.04 μm membranes) had a total membrane surface area of ~ 300 cm². After the adhesive dried, membranes were soaked in DI water for at least 24 h before use.
3.2.3. Foulant preparation and particle size

Silica microspheres and humic acid (HA) were selected as model foulants. According to the manufacturer, the average particle size of spherical SiO$_2$ (99.998% purity, Nanostructured & Amorphous Materials) was in the 1 to 3.5 µm range. To prepare a feed suspension with silica, SiO$_2$ particles were added to 0.5 L of DI water, mixed for 1 h and then added to the feed tank. To prepare a feed suspension with HA, 12 g of HA (Aldrich) were added into 4 L of DI water in an amber jar and the pH was adjusted to 8. The solution was mixed using a magnetic stir bar for 72 h, and then filtered through 0.45 µm membrane filters (Millipore). Filtered HA solution was stored at 4°C until use. Total organic carbon (TOC) content of the feed water was measured using TOC analyzer (OI Analytical). Particle size distribution in the stock was measured using Mastersizer 2000 (Malvern).

3.2.4. Membrane filtration experiment

The schematic of the experimental unit is shown in Figure 3-1. The total volume of the reactor was 25 L. Diffusers were placed at the bottom of the feed tank to supply air and mix the feed water. Peristaltic digital pump (model 07523-80, MasterFlex L/S) was used to apply transmembrane pressure. Permeate flow rate and transmembrane pressure were measured using a digital flow meter (model 106-4-C-T4-C10, McMillan) and digital pressure sensor (Cole-Parmer, 68075-00), respectively. A LabView code was developed to record readings from the flowrate and pressure sensors and to control the flow rate of the pump.

Three experiments with feeds of different compositions were carried out with each type of hollow fiber membranes. Each experiment included 4 stages:

① Stage 1 (duration = 1 h). The feed tank, with air diffusers on the bottom, was filled with 18 L of DI water and 10 mL of HAdV 40 stock suspension was added to the DI water in the tank. Averaged over all experiments, the initial feed concentration of HAdV 40 in
the tank was 7.03 ± 0.32. The pH of the feed was adjusted to 7.

② Stage 2 (duration = 6 h). The transmembrane pressure was applied and filtration was carried out in a constant flux regime \( \dot{Q} = 50 \text{ mL/min}; \ j = 2.78 \cdot 10^{-5} \text{ m/s} \). Samples of feed and permeate were withdrawn periodically for qPCR analysis and calculation of HAdV rejection.

③ Stage 3 (duration = 8 h). Foulants were added to the feed tank. pH was adjusted to 7 again.

④ Stage 4 (duration = 6 h for UF membranes and 12 h for MF membranes). Transmembrane pressure was applied and the fouling test was carried out in constant flux regime \( \dot{Q} = 50 \text{ mL/min}; \ j = 2.78 \cdot 10^{-5} \text{ m/s} \). As in Stage 2, samples of feed and permeate were withdrawn periodically for qPCR analysis and calculation of HAdV rejection.

At each stage the feed water was mixed by continuous aeration.

Feed and permeate samples were taken when the flow rate reached the target value of 50 mL/min during Stage 2, and every 2 h afterward. All feed and permeate samples withdrawn from the feed tank during this stage were stored at -80 °C until DNA extraction. The sampling protocol for each experiment is detailed in Table 3-2. The high foulant concentrations were used to accelerate membrane fouling and shorten the time of data gathering.

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

Virus DNA in each sample was extracted using MagNa Pure Compact System automatic machine and Nucleic Acid Isolation Kits (Roche Applied Sciences). Carrier RNA (Qiagen) was used to enhance DNA recovery. The DNA extracts were placed into storage (-80 °C) immediately after extraction. Following DNA extraction, virus concentration was quantified using qPCR (Roche Light Cycler). Sequence of primers and TaqMan probe were adopted from Xagoraraki et al. (2007). Values of crossing point, \( C_p \), were automatically generated by
the LightCycler software. HAdV concentrations in feed and permeate sample were determined based on the $C_p$ values and the standard curve that was developed beforehand.

3.2.6. Inhibition of qPCR by humic acid

Sutlović et al. (2005) reported that polymerase chain reaction may be inhibited by HA. In order to evaluate the effect of qPCR inhibitors, we adapted the method of serial dilutions that was used by Ijzerman et al. (1997) and Gibson et al. (2012). A set of HA solutions with different concentrations of HA (0, 10, 20, 30, and 40 mg/L) were seeded with ~$10^7$ copies/mL of HAdV 40. Then DNA extraction and qPCR analysis were carried out to assess HA-induced inhibition of qPCR.

3.2.7. Scanning electron microscopy (SEM) imaging of membranes

SEM images of membrane skin surfaces and cross-sections were recorded using JSM-7500F microscope. When imaging skin surfaces of UF, MF1, and MF2 membranes, the magnifications were ×100,000, ×10,000, and ×2,200 respectively. Images of membrane cross-sections were taken with the magnification of ×170. SEM samples were prepared by immersing membrane coupons in liquid N₂, breaking them into smaller fragments, and mounting them on SEM aluminum stubs. MF1 and MF2 membranes were coated with ~14 nm Au layer in the Emscope sputter coater, while UF membranes were coated with ~4 nm Pt layer in the Electron Microscopy Sciences Q150T turbo-pumped coater.

3.2.8. Membrane challenge tests

To quantitative assess the retention ability of the membranes and supplement nominal pore size data provided by the manufacturers (Table 3-1), membrane challenge tests were performed using suspensions of monodisperse spherical probe particles. Fluorescent
polystyrene beads with the nominal diameter of 50, 100, 300, and 500 nm (PSF series) were purchased from Magsphere, Inc. The challenge tests were performed using the same filtration rig (except that a 1 L Nalgene bottle was used as a feed vessel) as in virus filtration studies (Section 2.2.4) and were run for 15 min at the constant permeate flow rate of 50 mL/min. For each probe/membrane combination, three permeate samples were collected 11, 13, and 15 min into the challenge test and the log removal (or rejection) value was calculated as an average for these three samples. Particle concentrations in the feed and permeate were determined spectrophotometrically (Multi-Spec 1501, Shimadzu). The absorbance was measured at $\lambda = 197, 202, 236,$ and $274$ nm with 50, 100, 300, and 500 nm probes, respectively.

3.3. Results and Discussion

3.3.1. Characterization of membranes and model foulants

Particle size distributions for SiO$_2$ suspension and solution of humic acid are shown in Figure 3-2. There was approximately an order of magnitude difference in size between the silica microspheres and humic acid aggregates. Values of $d_{0.1}$, $d_{0.5}$, and $d_{0.9}$ for the suspension SiO$_2$ particles were 1.81, 3.45, and 6.81 $\mu$m, respectively while for humic acid these values were 0.09, 0.15, and 0.33 $\mu$m.

Results of membrane challenge tests (Table 3-3) were consistent with the nominal pore sizes reported by the manufacturers (Table 3-1) and results of SEM imaging (Figure 3-3). As expected, larger probes were rejected more by all three membranes. The 100 nm probe, which was the closest to the size of HAdV-40, was rejected by UF, MF1 and MF2 membranes with rejections of 97.5%, 84.2% and 82%, which corresponded to LRV values of 1.61, 0.80, and 0.75 (Table 3-3).
3.3.2. Inhibition of qPCR by humic acid

HA-free and 10, 20, 30, and 40 mg (TOC)/l solutions of HA seeded with 10^7 copies/mL (i.e. 7 logs) of HAdV 40 were analyzed for virus concentration. In samples with 0, 10, 20, and 30 mg(TOC)/l, the concentration of HAdV was measured to be 7.02, 6.95, 6.93 and 6.82 logs, respectively. Only, in the 40 mg (TOC)/l solution the virus concentration could not be measured apparently because the fluorescence signal during Light Cycler measurements was inhibited by organic compounds in the sample. Given the negligible inhibition at sufficiently low HA concentrations, all feed samples were diluted ten-fold to adjust HA concentration to 4 mg/L. Additionally, permeate samples from experiments with 0.22 µm and 0.45 µm membranes treating the mixture of SiO₂ and HA were also diluted. As a control measure, all original and diluted samples were analyzed for comparison. Paired t-test showed significant (p < 0.05) difference between the original and adjusted concentrations in feed samples. In contrast, no significant difference was found for permeate samples. The dilution factor was taken into account during the virus removal calculation afterward.

3.3.3. Membrane fouling and transmembrane pressure buildup

Figure 3-3 illustrates changes in the transmembrane pressure with filtration time. During Stage 2 (filtration of HAdV 40 in DI water), the headloss increased slowly: \( \frac{dP}{dt} \) was only 0.05, 0.04, and 0.07 psi/h (345, 275, and 483 Pa/h) on average in filtration tests with UF \( d_{pore} = 0.04 \) µm, MF1 \( d_{pore} = 0.22 \) µm, and MF2 \( d_{pore} = 0.45 \) µm) membranes, respectively. This was due to the relatively low concentration of HAdV 40, the only foulant in the feed. Thus neither complete pore blocking (more likely to occur with the UF membrane), nor standard blocking (more likely to occur with the MF2 membrane) by HAdV 40 added much additional resistance to the permeate flow. In these constant flux experiments, the
permeate flow rate was maintained at 49.97 ± 1.60, 49.99 ± 1.44, and 49.17 ± 4.51 mL/min, respectively.

During Stage 4 of experiments with all three types of membranes, fouling by the mixture of HA and SiO₂ particles was significantly higher than the sum of contributions due to each of these two feed components fouling the membrane separately. For example, for the UF membrane \( d_{pore} = 0.04 \mu m \), the pressure increase rate during initial stages of fouling was ~ 1.3, 1.4, and 24.5 psi/h (9.0, 9.7, and 175.2 kPa/h) on average in tests with SiO₂, HA, and SiO₂/HA mixture, respectively. We hypothesize that the synergy stemmed from two separate but related effects. On the one hand, accumulation of SiO₂ particles on the membrane surface likely hindered back-diffusion of HA away from the membrane and resulted in more blockage of membrane pores by HA. On the other hand, such accumulation of HA near the membrane in the presence of a SiO₂ cake, could lead to the formation of a composite SiO₂/HA layer with a higher specific hydraulic resistance than that of the cake composed of SiO₂ particles only. Consistent with the above hypothesis is the observation that the mutual enhancement of fouling by SiO₂ and HA was particularly evident in experiments with the UF membrane; indeed, HA should be more effective in blocking smaller pores of this membrane.

To confirm that pore blockage and cake formation were indeed operative fouling mechanisms, we performed a separate set of constant pressure dead-end filtration experiments and applied blocking laws (Hermia 1982) to the permeate flux data generated in these tests. The tests were performed in the absence of aeration to satisfy assumptions behind the blocking law theory. The theory’s assumption that all membrane pores are of the same size was not met though: UF, MF1, and MF2 are phase inversion membranes with complex pore space morphology and a distribution of pore sizes. Thus, the model’s predictions are only approximations. Figure 3-5 presents an example of filtration data (Stage 4; see Section 3.2.4) in the \( \frac{d^2t}{dv^2} \) vs \( \frac{dt}{dv} \) format where three segments corresponding to pore blockage (0
< \( n < 2 \) > < \( n < 0 \) > and cake filtration (= 0) and the transition between these two regimes (\( n < 0 \)) could be discerned. However, it was also clear that applying one of the three blocking laws does not provide a complete description of the fouling process as segments of the \( \frac{d^2 t}{dV^2} \) vs \( \frac{dt}{dV} \) dependence had negative slope not accounted for by the model. The negative slope is explained by the combined pore blockage-cake filtration model (Ho and Zydney, 2000) as resulting from the simultaneous pore blockage and formation of the cake over blocked areas of the membrane.

The observed negative slope is consistent with the findings of Yuan et al. (2002) who suggested that membrane fouling by humic acid is a combined effect of pore blockage and cake layer formation.

While the slope for the MF2 membranes was somewhat variable and deviated from zero, the \( \frac{d^2 t}{dV^2} \) vs \( \frac{dt}{dV} \) dependence for MF2 clearly followed a different trend and was “flatter” than that recorded for the UF membrane. Figure 3-5 confirms that the main mechanism of fouling by SiO\(_2\) was cake filtration and that pore blockage was one of fouling mechanisms during UF filtration of HA-containing feed waters.

3.3.4. Removal of human adenovirus 40 by clean and fouled membranes

As shown in Figure 3-6, the removal of virus suspended in DI water (Stage 1) by UF (\( d_{\text{pore}} = 0.04 \mu m \)), MF1 (\( d_{\text{pore}} = 0.22 \mu m \)) and MF2 (\( d_{\text{pore}} = 0.45 \mu m \)) membranes was 2.27 log, 0.70 log and 0.73 log, respectively. Student’s t-test showed that virus removal by the UF membrane was significantly (\( p < 0.05 \)) higher than by each of the MF membranes, while there was no significant difference in virus removal between MF1 and MF2. The standard deviations reflect the variability in virus rejection with time.
Given that the hydrodynamic diameter of HAdV 40 is ~ 0.1 μm, the results are consistent with size exclusion as a removal mechanism. That HAdV 40 is not completely removed by the UF membrane can be attributed to the finite width of the pore sizes distribution of the membrane. In a survey that included 27 membranes and two phages (Qâ and T4), Urase et al. (1996) reported incomplete removal of viruses for all membranes (including track etched and other narrow pore distribution ultra- and microfilters) and attributed this result to the presence of abnormally large pores.

Figure 3-7 describes virus removal by UF, MF1, and MF2 in the presence of foulants in the feed. Values of log removal of HAdV 40 from DI water (Stage 2) are shown next to removal values from Stage 4 of the same experiment. We also note that removal data in Figure 3-6 are averages of the values of HAdV removal from DI water as reported in Figure 3-7; for example, the LRV value of 2.27 given in Figure 3-6 for the UF membrane is the average of the three values (LRV = 2.23, 1.96, and 2.62) reported in Figure 3-7a.

Virus removal by UF membrane \(d_{\text{pores}} = 0.04 \, \mu m\) was enhanced in the presence of HA as the only foulant but was reduced when SiO\(_2\) particles were the sole foulant (Figure 3-7a). These opposite effects can be tentatively attributed to two different mechanisms. The first mechanism is the partial or complete blockage of membrane pores by HA. Partial blockage decreases effective pore size while the pores that are “completely blocked” may allow permeation of water but not virus passage. (Incidentally, the fouling model developed by Ho and Zydney (2000) allows some fluid flow through blocked pores.) Either scenario – be it partial or complete blockage – leads to improved virus removal. Competition for the adsorption sites on the membrane surface could be a contributing factor: previous studies have demonstrated that HA inhibits virus adsorption onto membranes and decreases their ability to retain viruses during filtration (Zheng et al., 2005; Zheng and Liu, 2007). The second mechanism is the possible increase of the transmembrane differential in virus concentration due to cake formation. The latter mechanism is consistent with the hypothesis
proposed in Section 3.3.3 where permeate flux decline was interpreted in terms of the cake-enhanced concentration polarization and accumulation of HA in the pores of both the membrane and the SiO₂ layer. The cake composed of larger SiO₂ particles is too porous to effectively reject HAdV 40 yet may be sufficiently dense to hinder HAdV 40 back-diffusion leading to its accumulation near the cake-membrane interface. The higher transmembrane differential of virus concentration leads to enhanced virus transport across the membrane and lower virus removal. When both foulants are present in the feed, the two opposing effects appear to cancel each other (Figure 3-7a).

In experiments with MF1 (dₚore = 0.22 µm) the effects of the two types of foulants acting alone and in combination were similar to but less pronounced than those observed in UF tests (Figure 3-7b). In experiments with MF2 membrane (dₚore = 0.45 µm), however, neither HA alone nor SiO₂ alone had statistically significant effects on HAdV 40 removal (Figure 3-7c). HA molecules were not large enough to block membrane pores effectively; indeed, HA was pre-filtered through a 0.45 µm membrane prior to virus removal tests (see Section 3.2.3). Formation of the SiO₂-only cake lead to less significant cake-enhanced accumulation of the virus and had only a slight effect on virus removal by MF2 membranes. This was because the effective pore size of the cake was closer to that of the MF2 skin (than to that of UF and MF1 skins); thus, fouling of MF2 by SiO₂ effectively increased the thickness of the membrane, added a small additional resistance to the permeate flow (Figure 3-4c) but did not significantly increase virus concentration at the membrane surface.

Combined fouling of MF2 by HA and SiO₂ lead to a statistically significant decrease in HAdV 40 removal (Figure 3-7c). Apparently the composite HA/SiO₂ cake was dense enough to capture and concentrate viruses for higher transmembrane concentration differential and enhance virus transport across the membrane; at the same time, in contrast to UF and MF1, MF2 membrane pores were too large to be blocked by HA and reject viruses. Figure 3-8
schematically illustrates how the two hypothesized effects of fouling on virus removal manifest themselves in UF (Figure 3-8; A, B, C) and MF (Figure 3-8; D, E, F):

- Figure 3-8 A, C: increased virus removal due to pore blockage by dissolved species (also see Figure 3-7a (HA and HA+SiO$_2$ data));
- Figure 3-8 B, C: decreased removal due to cake-enhanced accumulation of virus near the membrane (also see Fig. 7a (SiO$_2$ and HA+SiO$_2$ data));
- Figure 3-8 C, F: additional removal by the composite HA/SiO$_2$ cake (also see Figure 7c (HA+SiO$_2$ data)).

It is very clear from Figure 3-7 and Figure 3-4 that the extent of fouling or rate of fouling increase are not reliable predictors of virus removal, which can either increase or decrease as a result of membrane fouling. Instead, feed water composition and membrane pore size together govern virus removal with fouling mechanisms playing a key mediating role: pore blockage improves virus removal while cake formation can either increase or decrease virus removal depending on the permeability of the cake.

3.4. Conclusions

This study demonstrates that membrane fouling may have a profound impact on HAdV 40 removal by membranes. In the absence of fouling, average values of HAdV 40 removal by hollow fiber ultrafiltration (UF, $d_{pore} = 0.04$ μm) and microfiltration (MF1, $d_{pore} = 0.22$ μm; MF2, $d_{pore} = 0.45$ μm) membranes are 2.3 log, 0.7 log and 0.7 log, respectively. Fouling by humic acid (model dissolved species), SiO$_2$ microspheres (model suspended species) and a mix of these constituents alters membrane’s ability to remove HAdV. Three separate effects are identified: 1) increased removal due to pore blockage by dissolved species; 2) decreased removal due to cake-enhanced accumulation of viruses near membrane
surface; and 3) increased removal by the composite cake acting as a secondary membrane.

The results indicate that the extent of fouling is not a reliable predictor of virus removal. Instead, feed water composition and membrane pore size together govern virus removal with fouling mechanisms playing a key mediating role: pore blockage improves virus removal while cake formation can either increase or decrease virus removal depending on the relative permeability of the cake.
Table 3-1. Characteristics of hollow fiber membranes

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Notation</th>
<th>Manufacturer</th>
<th>Material</th>
<th>Nominal pore size, µm</th>
<th>Outer diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF</td>
<td>General Electric</td>
<td>Polyvinylidene fluoride</td>
<td>0.04</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>MF1</td>
<td>Shenzhen Youber Technology</td>
<td></td>
<td>0.22</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>MF2</td>
<td></td>
<td></td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-2. Sampling protocols in fouling experiments with different membranes and feed waters of different compositions

<table>
<thead>
<tr>
<th>Test type</th>
<th>Foulant concentration</th>
<th>Experiment duration, h (Time between samples, h) in tests with different membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>800 mg (SiO$_2$)/L</td>
<td>$d_{pore} = 0.04 \mu m$ 6 (2) 12 (4) 12 (4)</td>
</tr>
<tr>
<td>HA</td>
<td>40 mg (HA)/L</td>
<td>$d_{pore} = 0.22 \mu m$ 6 (2) 12 (4) 12 (4)</td>
</tr>
<tr>
<td>SiO$_2$, HA</td>
<td>800 mg (SiO$_2$)/L</td>
<td>$d_{pore} = 0.45 \mu m$ 0.5 (0.08) 3 (1) 2 (0.5)</td>
</tr>
<tr>
<td></td>
<td>40 mg (HA)/L</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-3. Log removal of probe particles in challenge tests with the UF, MF1, and MF2 membranes

<table>
<thead>
<tr>
<th>Diameter of the probe particle, nm</th>
<th>Membrane type (nominal pore size)</th>
<th>$d_{\text{pore}} = 0.04 \mu m$</th>
<th>$d_{\text{pore}} = 0.22 \mu m$</th>
<th>$d_{\text{pore}} = 0.45 \mu m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1.16 ± 0.01</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>100</td>
<td>1.61 ± 0.01</td>
<td>0.80 ± 0.02</td>
<td>0.75 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>1.86 ± 0.10</td>
<td>0.89 ± 0.02</td>
<td>0.86 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>n/a</td>
<td>1.43 ± 0.23</td>
<td>0.95 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3-1. Schematic of the experimental apparatus

Figure 3-2. Particle size distribution of model foulants
Figure 3-3. SEM micrographs of cross-sections (A - C) and the planar view of the separation layer (D – F) of the three membranes

*UF with* \( d_{\text{pore}} = 0.04 \, \mu \text{m} \) (A, D); *MF1 with* \( d_{\text{pore}} = 0.22 \, \mu \text{m} \) (B, E); *and MF2 with* \( d_{\text{pore}} = 0.45 \, \mu \text{m} \) (C, F)
**Figure 3-4.** Transmembrane pressure as a function of time during filtration of HAdV 40 suspension (-○-, -□-, -△-) and HAdV-seeded feeds containing SiO₂ microspheres (-●-), humic acid (-■-), and SiO₂/HA mixture (-▲-) by three membranes of different nominal pore sizes: a) 0.04 µm, b) 0.22 µm, and c) 0.45 µm*

*Circled numbers mark experimental stages (see Section 3.2.4)*
Figure 3-5. Blocking laws applied to filtration of SiO$_2$ microspheres and humic acid by UF and MF2 membranes
Figure 3-6. Removal of HAdV 40 from DI water by three membranes of different nominal pore sizes*.

*UF ($d_{pore} = 0.04 \mu m$), MF1 ($d_{pore} = 0.22 \mu m$), and MF2 ($d_{pore} = 0.45 \mu m$). The values represent averages over the duration of Stage 2 of the experiment (see Section 3.2.4) and over all experiments with a membrane of a given pore size.
Figure 3-7. Comparison of HAdV 40 removal from DI water, suspension of SiO$_2$ microspheres, solution of humic acid, and SiO$_2$/HA mixture by three membranes of different nominal pore sizes: a) 0.04 µm, b) 0.22 µm, and c) 0.45 µm.*

*The values represent averages over the duration of Stage 4 of the experiment (see Section 3.2.4).
**Figure 3-8.** Schematic illustration of effects of fouling on HAdV 40 removal by ultrafiltration (A, B, C) and microfiltration (D, E, F) membranes under conditions of fouling by dissolved species (A, D), suspended particles (B, E) and by both of these foulants (C, F)*

*HAdV 40, dissolved species and suspended species are depicted as blue dots with spikes, orange random shapes, and gray spheres, respectively. Average values of log removal of HAdV 40 by clean UF and MF2 membranes are 2.27 and 0.73, respectively.*
REFERENCES
REFERENCES


CHAPTER 4

EFFECT OF PRESSURE RELAXATION AND MEMBRANE BACKWASH
ON VIRUS REMOVAL IN A MEMBRANE BIOREACTOR

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 ($t_{\Delta P>0}$), pressure relaxation ($t_{\Delta P=0}$) and backwash ($t_{\Delta P<0}$) steps. Both pressure relaxation and permeate backwash led to significant reductions in virus removal. For the same value of $t_{\Delta P>0}/t_{\Delta P=0}$, longer filtration/relaxation cycles (i.e. larger $t_{\Delta P} + t_{\Delta P=0}$) led to higher transmembrane pressure ($\Delta P$) but did not have a significant impact on virus removal. A shorter backwash ($t_{\Delta P<0} = 10$ min) at a higher flow rate ($Q = 40$ mL/min) resulted in more substantial decreases in $\Delta P$ and virus removal than a longer backwash ($t_{\Delta P<0} = 20$ min) at a lower flow rate ($Q = 20$ mL/min) even though the backwash volume ($Qt_{\Delta P<0}$) was the same. Virus 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 $\Delta P$ and virus removal.

Keywords: membrane bioreactor, membrane fouling, human adenovirus, pressure relaxation, backwash
4.1. 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). 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).

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). 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 five 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 Xagoraraki 2011). (Zanetti et al. 2010) measured LRVs for F-specific coliphage and somatic coliphage to be 6 logs and 4 logs, respectively.

The role of biofilm in virus removal by MBRs has been studied by (Wu et al. 2010) who found that the clean membrane ($d_{pore} = 0.4 \mu m$) 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 \mu m 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 4-1). Most of the published work on the subject focused on chemical cleaning and employed bacteriophages.

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.

To our knowledge, the impacts of pressure relaxation, air scouring and permeate backwash on virus removal in MBR systems have not been investigated yet. 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.

4.2. Materials and Methods

4.2.1. Cell culture experiment and virus incubation

A549 cell line has been suggested as an efficient cell line for HAdV (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).

4.2.2. 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 (90 cm long each) in a short (~10 cm) piece of 1/2” ID PFTE tubing with an adhesive (Loctite). Each membrane unit had an effective surface area of ~1600 cm², and 4 such units were used in each experiment. 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.
4.2.3. Bench-scale submerged MBR

A schematic of the bench-scale MBR system is shown in Figure 4-1. 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 \( Q = 31.3 \text{ mL/min}; \ j = 3.26 \times 10^{-6} \text{ m/s} \). Transmembrane pressure \( \Delta P \) 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 4-2) 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).

4.2.4. 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 4-3. All samples were stored at -80 °C until analysis.

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 4.2.3. 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 4-3. Two sets of samples were collected every 16 h, following the same sampling strategy as in Stage 1.

4.2.5. 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 GCCACCGTGGGGTTTTCTAAACTT, 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.

4.2.6. 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.

4.3. Results and Discussion

4.3.1. Membrane fouling and transmembrane pressure buildup

Figure 4-2 summarizes ΔP 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 4-3). The LabView program was used to log in ΔP data every second. Each dot in Figure 4-2 represents the average value of ΔP 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, ΔP
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) \(\Delta P\) 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 \(t_{\Delta p=0}\) to filtration time \(t_{\Delta p>0}\) 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 \(t_{\Delta p=0}\) 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 \(\Delta P\). In exp.1, backwash was conducted at 40 mL/min rate for 10 min, and the \(\Delta P\) decreased by 3.6 kPa and 2.5 kPa in Stage 2 and 3, respectively. In exp. 2 with 20 min backwash at 20 mL/min rate, the \(\Delta P\) decreased by 2.6 kPa and 1.6 kPa in Stage 2 and 3, respectively. With the same backwash flow rate and duration, \(\Delta P\) in exp. 3 dropped by 3.5 kPa at Stage 2, which is similar to what was observed in exp. 1. However, \(\Delta P\) 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 \(\Delta P\). 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 $\Delta P$ 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).

4.3.2. Virus 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 4-3). 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, (Xagoraraki 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. In a 35-h experiment with a pilot-scale side-stream MBR, the removals of nitrate, total organic carbon and alachlor sharply increased in the first 5 – 10 hours and then remained relatively constant during the rest of the experiment (Ravindran et al. 2009). 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 flow rate of 40 mL/min 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 flow rate of 20 mL/min for 20 minutes. As a result, backwash 1 lowered LRV by 0.33 and 0.31 log while backwash 2 barely affected the LRV.

Reduction of virus removal caused by different backwash formats is summarized in Figure 4-4, 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 ΔP 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) 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 $\Delta P$. 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. Moreover, it is notable that in all sampling events, virus 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 can enhance virus removal. Our previous study indicates that the reversible fouling caused by silica particles (3.45 µm in diameter) reduced virus removal, especially in the case small pore size 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 4-5, 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, $\Delta P$ increase is caused by reversible fouling. In each filtration cycle
after the flow rate reached a constant level, the change in $\Delta P$ was very slow. This is supported by the t-test which showed the difference of $\Delta P$ 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 $\Delta P$ did not increase further in each filtration cycle despite the fact that duration of filtration cycles was doubled.

4.3.3. Relationship between transmembrane pressure and virus removal

Figure 4-6 demonstrates the correlation between transmembrane pressure ($\Delta P$) and virus removal (LRV). $\Delta P$ and LRV in exp. 1 and exp. 2 were moderately correlated ($R^2 = 0.63$ and $0.78$, respectively) to each other, while a relatively strong correlation ($R^2 = 0.94$) was observed in exp. 3. A moderate correlation ($R^2 = 0.72$) was obtained when the analysis was applied to data from all three experiments. The correlations found in our study are stronger than those observed by (Wu et al. 2010), who reported a moderate correlation ($R^2 = 0.656$) between $\Delta P$ and LRV of indigenous somatic coliphages. The possible correlation between $\Delta P$ and LRV was also explored by (Shang et al. 2005), but these authors suggested that the correlation only exists when the food to mass ratio is low. In sum, $\Delta P$ may be used to estimate levels of virus removal in MBR systems, and higher $\Delta P$ generally leads to greater virus removal. However, the quantitative correlations may be system-dependent and vary with the virus type.

4.4. Conclusions

This study demonstrated the change of transmembrane pressure (TMP) and removal of human adenovirus, when periodical pressure relaxation and permeate
backwash were applied in a bench-scale MBR. Based on the data presented above, following conclusions can be drawn:

- Both pressure relaxation and permeate backwash can mitigate membrane fouling, and meanwhile decrease virus removal. Reversible fouling plays an important role in removing viruses in MBRs.
- With same permeate volume for backwash, higher backwash flux can cause more reduction in TMP and virus removal.
- With same filtration/relaxation ratio, longer cycle will lead to higher extent of fouling, but its impact on virus removal is not significant.
- TMP may be used to estimate level of virus removal in MBRs. Higher TMP generally leads to greater virus removal. But the quantitative correlation between TMP and virus removal is not very persistent.
APPENDIX
<table>
<thead>
<tr>
<th>MBR</th>
<th>$d_{	ext{pore}}$</th>
<th>Fouling mitigation method</th>
<th>Effect on fouling</th>
<th>Virus</th>
<th>Effect on virus removal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench</td>
<td>0.4 µm</td>
<td>Chemical backwash</td>
<td>NA</td>
<td>Somatic coliphage</td>
<td>$\Delta LRV \leq 1^\circ$</td>
<td>Wu et al. 2010</td>
</tr>
<tr>
<td>scale</td>
<td>0.4 µm</td>
<td>Chemical backwash Original pressure recovered</td>
<td>MS -2 coliphage</td>
<td></td>
<td>0.4 &lt; $\Delta LRV \leq 2.5$</td>
<td>Shang et al. 2005</td>
</tr>
<tr>
<td></td>
<td>0.1 µm</td>
<td>Chemical clean</td>
<td>NA</td>
<td>F-specific coliphage</td>
<td></td>
<td>Tam et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Tap water flush</td>
<td></td>
<td></td>
<td>MS -2 coliphage</td>
<td></td>
<td>Hirani et al. 2014</td>
</tr>
<tr>
<td>Pilot</td>
<td>0.1 µm and 0.22 µm</td>
<td>Tap water flush chemical clean</td>
<td></td>
<td>T4 coliphage</td>
<td></td>
<td>Zheng et al. 2005</td>
</tr>
<tr>
<td>scale</td>
<td>0.1 µm</td>
<td>Tap water flush chemical clean</td>
<td></td>
<td></td>
<td>LRV further decreased to 4 logs</td>
<td>Lv et al. 2006</td>
</tr>
<tr>
<td>Bench</td>
<td>0.22 µm</td>
<td>Tap water flush chemical clean</td>
<td></td>
<td>T4 coliphage</td>
<td>LRV further decreased to 1.9 logs</td>
<td>Lv et al. 2006</td>
</tr>
<tr>
<td>scale,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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Table 4-1. Effect of membrane fouling mitigation methods on virus removal in submerged MBRs
<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Daily dose (g)</th>
<th>Chemicals</th>
<th>Daily dose (mg)</th>
</tr>
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<tr>
<td>Glucose</td>
<td>15</td>
<td>H\textsubscript{3}BO\textsubscript{3}</td>
<td>3.60</td>
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<tr>
<td>Peptone</td>
<td>5</td>
<td>CuSO\textsubscript{4} \cdot 5H\textsubscript{2}O</td>
<td>0.72</td>
</tr>
<tr>
<td>KH\textsubscript{2}PO\textsubscript{4}</td>
<td>1.6</td>
<td>KI</td>
<td>5.12</td>
</tr>
<tr>
<td>(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}</td>
<td>5.2</td>
<td>MnCl\textsubscript{2} \cdot 4H\textsubscript{2}O</td>
<td>2.88</td>
</tr>
<tr>
<td>MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O</td>
<td>3.2</td>
<td>NaMoO\textsubscript{4} \cdot 2H\textsubscript{2}O</td>
<td>1.44</td>
</tr>
<tr>
<td>CaCl\textsubscript{2} \cdot 2H\textsubscript{2}O</td>
<td>1.6</td>
<td>ZnSO\textsubscript{4} \cdot 7H\textsubscript{2}O</td>
<td>2.88</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.24</td>
<td>CoCl\textsubscript{2} \cdot 6H\textsubscript{2}O</td>
<td>3.60</td>
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<tr>
<td>NaCl</td>
<td>5</td>
<td>FeCl\textsubscript{3} \cdot 6H\textsubscript{2}O</td>
<td>36.00</td>
</tr>
</tbody>
</table>

* 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).
Table 4-3. Parameters of pressure relaxation and backwash

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Pressure relaxation (ΔP = 0)</th>
<th>Permeate backwash (ΔP &lt; 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_{ΔP&gt;0}$ / $t_{ΔP=0}$</td>
<td>Flow rate</td>
</tr>
<tr>
<td>1</td>
<td>25 min / 5 min</td>
<td>40 mL/min</td>
</tr>
<tr>
<td>2</td>
<td>25 min / 5 min</td>
<td>20 mL/min</td>
</tr>
<tr>
<td>3</td>
<td>50 min / 10 min</td>
<td>40 mL/min</td>
</tr>
</tbody>
</table>
Figure 4-1. Schematic of the submerged MBR
Figure 4-2. Transmembrane pressure as a function of filtration time and the effect of backwash
Figure 4-3. 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: A: exp. 1; B: exp. 2; C: exp. 3
Figure 4-3. (Cont’d)
Figure 4-4. Decrease in virus removal as a result of backwash for two different backwash formats.
Figure 4-5. Decrease in virus removal as a result of pressure relaxation for two different formats of the filtration/relaxation (F/R) cycle.
**Figure 4-6.** Correlations between virus removal and transmembrane pressure in experiments 1 (A), 2 (B) and 3 (C)*

*For each data point, $\Delta P$ represents the pressure averaged over 11 s interval around the corresponding sampling point*
Figure 4-6. (Cont’d)

\[ LRV = 0.2136\Delta P(kPa) + 1.4246 \]

\[ R^2 = 0.9366 \]
REFERENCES
REFERENCES


Lee, C., Lee, S. H., Han, E., & Kim, S. J. (2004). Use of cell culture-PCR assay based on combination of A549 and BGMK cell lines and molecular identification as a...
tool to monitor infectious adenoviruses and enteroviruses in river water. Applied and Environmental Microbiology, 70(11), 6695-6705.


CHAPTER 5

ADSORPTION AND DESORPTION OF HUMAN ADENOVIRUS TO PRIMARY AND SECONDARY SLUDGE

Abstract

The presence of human enteric viruses in water, and their resulting potential to cause diseases, posed a threat on public health. Virus adsorption to sludge particles has been suggested as one of the major mechanisms of virus removal, while the studies focused on sorption kinetics of viruses in sludges are limited. With assistance of real-time quantitative polymerase chain reaction (qPCR), we explored the adsorption and desorption of human adenovirus 40 (HAdV) in primary and secondary sludge. The results showed that greater HAdV adsorption was observed when sludge filtrate was used as solute compared to DI water. Adsorption of HAdV conformed to Freundlich isotherm, and it exhibited very similar behavior in the two types of sludge. Desorption of HAdV from sludge particles was not very significant in sequential desorption experiments. More HAdV was desorbed from primary sludge than from secondary sludge, but the difference was not statistically significant.

Key words: adsorption, desorption, human adenovirus, Freundlich isotherm
5.1. Introduction

5.1.1. Viruses in the wastewater

Enteric viruses pose a considerable threat to human health due to their low infectious dose and long survival in the environment. Viruses have been noted in the contaminant candidate lists (CCL) issued by U.S. Environmental Protection Agency (USEPA), including adenovirus, enterovirus, coxsackievirus, echovirus, hepatitis A virus, and calicivirus (Xagoraraki et al. 2014). A large number of enteric viruses are excreted in human feces and urine, which makes wastewater one of the most concentrated sources of viruses (Puig et al. 1994, Castignolles et al. 1998). It has been reported that virus concentration in wastewater could be up to \(10^9\) copies per liter (da Silva et al. 2007; Kuo et al. 2010; Simmons et al. 2011). Therefore, it is critical to remove viruses from wastewater before discharging it into the environment. However, wastewater treatment systems may not be able to serve as an absolute barrier against contaminants, and the presence of enteric viruses are frequently reported in treated wastewater, even in the effluent from membrane bioreactors (MBR), the most advanced wastewater treatment systems (Xagoraraki et al. 2014; Simmons et al. 2011; Kuo et al. 2010). Wastewater effluents have been considered as one of major potential sources of pathogens (Bitton and Harvey 1992). Sludge, containing viruses, from wastewater treatment process is likely to be applied to landfills, and biosolids (treated sludge) containing viruses, may be applied on agricultural land (Wong et al. 2010).

5.1.2. Virus sorption mechanisms

Transport and survival of viruses in the environment is largely controlled by adsorption and desorption. Conventional activated sludge is the most widely used wastewater treatment system worldwide, where adsorption to biosolids is the one of
the major mechanisms for virus removal (Gerba et al. 1975; Vilker et al. 1980; Gerba 1984; Kim and Uno 1996). Rainfall may cause virus desorption from land applied biosolids and contaminate groundwater, which is one of the major sources of drinking water. When viruses come to the surface water systems, adsorption to suspended particles may facilitate virus survival and transport, while soil or sediment can serve as a reservoir and shade for viruses to survive (Gerba and Schaiberger 1975; Hurst et al. 1980). Consequently, understanding virus adsorption and desorption behavior in different environmental circumstances is a key step to prevent people from exposure to pathogenic viruses.

Virus adsorption is type and strain specific (Boche and Quilligan 1966; Goyal and Gerba 1979; Gerba et al. 1980; Gerba et al. 1981). Larger virus size facilitates adsorption due to more available surface charges (Dowd et al., 1998; Chattopadhyay and Puls, 1999). Surface charge is an important factor for virus adsorption, and the attractive forces between sorbents and viruses tend to be stronger if they have opposite surface charge. Hydropobicity is widely used to explain different affinity between different types of viruses and sorbents (Ivanova et al. 2011; Han et al. 2006; Bales et al. 1991). It has been concluded that hydrophobic sorbents are more favored to adsorb hydrophobic viruses, and vice versa (Chattopadhyay et al. 2002). Additionally, the forming of hydrogen and/or hydroxyl bond between sorbents and viruses has been suggested as an important factor enhancing adsorption (Oza and Chaudhuri 1975; Oza and Chaudhuri 1976).

Water content, also known as moisture content, refers to the amount of water contained in a material. An agreement appears to be reached among previous literature that virus adsorption is enhanced at lower water content in all kinds of sorbents (Powelson et al. 1990; Jin et al. 2000; Zhao et al. 2008; Han et al. 2006; Yeager and O’Brien 1979; Poletika et al. 1995; Chu et al. 2001; Chu et al. 2003).
Three mechanisms have been proposed for this phenomenon: (1) adsorption is promoted in the solid-water interface of media with lower water content due to higher extend of proximity between viruses and solid surface (Preston and Farrch 1988; Bitton et al. 1984); (2) the air-water interface (AWI), which only exists in unsaturated media, can provide additional sorption sites for colloid particles to attach (Wan and Wilson 1994; Powelson and Mills 1996; Jewett et al. 1999); (3) water content can also influence virus transport by film straining effect that transport of colloidal particles is restricted in porous media when the thickness of water film is smaller than the particle diameter (Wan and Tokunaga 1997; Han et al. 2006).

Greater virus adsorption to sorbents is usually observed at lower pH (Schulze-Makuch et al. 2003; Chaudhuri et al. 1977; Drewry and Eliassen 1968; Zhao et al. 2008; Oza and Chaudhuri 1976; You et al. 2003; Bales et al. 1993 and 1995), but not all sorption experiments from previous studies followed the same trend (Cookson 1969; Oza and Chaudhuri 1975). pH can govern virus sorption by altering the surface charge. For example, soils and viruses are usually negatively charged in natural environment (Oze and Chaudhuri 1976; Bitton 1975). With increased pH, the charge on both virus and the sorbent surfaces becomes more negative, between which the adsorption becomes weaker due to increased electrostatic repulsion among each other (Zhao et al. 2008; Drewry and Eliassen 1968; Chaudhuri et al. 1977). The Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, also known as double layer theory, is frequently employed to explain the impact of pH on virus sorption: when pH is increased, the diffused layer becomes thinner, and surfaces of viruses and sorbents have greater opportunities to approach each, where the van der Waals attraction becomes more significant (Gerba 1984). Moreover, tail fibers on virus surface are thought to attach on sorbent, and they are extended for adsorption at pH between 6.0 and 9.5. Otherwise, they will attach to tail sheath, and the viral
adsorption is weakened (Cookson 1969).

Higher ionic strength usually leads to greater virus adsorption (Carlson et al. 1968; Chaudhuri et al. 1977; Drewry and Eliassen 1968; Cao et al. 2010; Bradley et al. 2011; Oza and Chaudhuri 1976; Preston and Farrah 1988; Grant et al. 1993; Lance and Gerba 1984; Lipson and Stotzky 1983; Pham et al. 2009; Wallis and Melnick 1967), but not always (Chu et al. 2000; Zhuang and Jin 2003a; Cookson 1969; Penrod et al. 1996; Thompson and Yates 1999; Bales et al. 1993). Three mechanisms have been proposed: (1) according to DLVO theory, the electric double layer around viruses and sorbents will be squeezed due to high ionic strength, and those colloidal particles have greater chance to get close to each other, which leads to more adsorption (Chu et al. 2000; Lance and Gerba 1984; Lance et al. 1976; Bitton 1975); (2) the salt ions in aqueous solutions shield surfaces of viruses and sorbents and prevent them from interacting with each other; (3) virus fibers extend for sorption at low ionic strength, and attach to tail sheath when the ionic strength is high (Cookson 1969).

It has been well accepted that organic matter inhibits virus adsorption and enhances desorption (Carlson et al. 1968; Powelson et al. 1991; Lance and Gerba 1984; Lipson and Stotzky 1984; Stagg et al. 1977; Bixby and O'Brien 1979; Guttman-Bass and Catalano-Sherman 1986; Wong et al. 2013; Lo and Sproul 1977; Pham et al. 2009; Scheuerman et al. 1979; Bales et al. 1993; Cliver 1968; Ryan et al. 1999). Competition between organic material and virus particles for sorption sites appears to be the most popular mechanisms for the inhibition of viral adsorption (Carlson et al. 1968; Powelson et al. 1991; Zhuang and Jin 2003a; Bixby and O'Brien 1979; Lo and Sproul 1977; Pieper et al. 1997). It also has been suggested that organic material decreases virus adsorption by modifying surfaces of viruses and sorbents, or forming inert complex with viruses (Zhuang and Jin 2003a; Bixby and O'Brien 1979).
5.1.3. Virus sorption in activated sludge and biosolids

Adsorption to sludge particles has been suggested as the major mechanism of virus removal in wastewater treatment processes (Gerba et al. 1975; Vilk et al. 1980; Gerba 1984). Virus partitioning and removal due to sorption in activated sludge is summarized in Table 5-1. Enteroviruses showed higher adsorptive affinity to activated sludge than rotaviruses (Farrah et al. 1978). Balluz et al. (1978) reported that virus (f2 coliphage) distribution in liquid and solid phase was at the ratio of 18:82 in activated sludge. Rao et al. (1987) observed that 92% spiked rotavirus was attached to suspended solids in activated sludge. Similar results were obtained by Englande et al. (1983), who collected samples from multiple municipal wastewater treatment plants, and demonstrated the majority of viruses (mostly > 90%) were associated with solids. Moore et al. (1978) observed approximately 83 - 99% of the indigenous enteroviruses were attached to solids. Virus partition between solid and liquid phase is determined by adsorption capacity of activated sludge (Arraj et al. 2005).

Presence of adenoviruses, enteroviruses and noroviruses in dewatered sludge and class B biosolids has been previously reported, of which the concentration could be up to 10^8 copies per gram (Bofill-Mas et al. 2006; Monpoeho et al. 2001 and 2004; Viau and Peccia 2009; Wong et al. 2010). Adsorption is a reversible process, which depends on temperature, pH, ionic strength, soil properties and virus type and amount (Jørgensen and Lund 1986). Virus detachment from biosolids is of concern because more than half of biosolids generated in the United States are applied to landfills (NRC 2002). Change of environmental conditions, such as rainfall, may enhance desorption of virus and cause contamination (Engelnd et al. 1983; Landry et al. 1980). Desorption of viruses from sludge/biosolids is present in Table 5-2.

Adenoviruses are double-stranded DNA viruses (Group I of Baltimore classification). Its virion size ranges from 70 to 140 nm in diameter, and the isoelectric
point (IEP) ranges between 3.5 and 4.5 (Xagoraraki et al. 2014). Removal of adenoviruses in full-scale wastewater treatment plants has been investigated in previous studies. In conventional wastewater treatment plants, the reported removal of adenoviruses ranged from 1.3 logs to 2.4 logs (Haramoto et al. 2007; Hewitt et al. 2011; Katayama et al. 2008), while in MBR systems, the removal has a higher range from 3.4 logs to 6.3 logs (Kuo et al. 2010; Simmons et al. 2011; Simmons and Xagoraraki 2011).

Most previous studies in this field emphasized the overall virus removal during the wastewater treatment process. While the sorption kinetics of viruses in sludge has been demonstrated by only a few studies (Clarke et al. 1961; Vilker et al. 1980) and the mechanisms of virus sorption in activated sludge are rarely illustrated. In order to accurately describe the fate of viruses in wastewater treatment systems, a better understanding of virus sorption and desorption is needed. Adsorption and desorption isotherms of human adenovirus (HAdV) have been established in soils (Wong et al. 2013), while partitioning of HAdV in activated sludge hasn’t been investigated yet. The objective of this study is to investigate adsorption and desorption of human adenovirus with sludge particles.

5.2. Material and Methods

5.2.1. Human adenovirus preparation

Human adenovirus 40 was selected for this study, and it was propagated in A549 cell lines (ATCC, VR-846). The detailed procedure of virus incubation was described in our previous study (Yin et al. 2015).
5.2.2. Sludge sampling and processing

Fresh primary and secondary sludge samples were collected from the wastewater treatment plant, East Lansing, MI, and kept at 4 °C before use. Since the primary sludge was very condensed, it was diluted by 30 times before processing. Measurement of total suspended solids (TSS) was conducted in duplicate by passing 40 mL well-mixed sludge sample through 0.45 µm filter, then incubating the filter at 108°C for 1 hour. Dissolved organic carbon (DOC) was measured using TOC analyzer (OI Analytical). The results are present in Table 5-3.

5.2.3. DNA extraction and qPCR assay

Extraction of virus DNA was implemented using MagNa Pure Compact System automatic machine and Nucleic Acid Isolation Kits (Roche Applied Sciences). Carrier RNA (Qiagen, Valencia, CA) was used to increase the efficiency of DNA recovery. The DNA was stored at -80 °C immediately after extraction. Quantification of virus was conducted in triplicate afterward using qPCR (Roche Light Cycler), of which the assay (sequence of primers and probe) were adopted from Xagoraraki et al. (2007). Crossing point (C_p) values were generated by the Light Cycler program. Virus concentrations were determined based on C_p values and previously developed standard curves. Inhibition test was conducted by spiking same amount of HAdV to liquid phage of sludge and DI water, and then measuring virus concentration by qPCR. No significant inhibition effect was found.

5.2.4. Equilibrium time determination

A rate study was conducted to determine time needed to reach equilibrium. Sludge sample was well mixed then diluted to the solid/liquid (S/L) ratio of 1:20000
as dry weight. 1 mL of virus solution (~ $10^7$) was added to 9 mL of diluted sludge and mixed by a tumbler at 20 rpm at room temperature. At the end of 12, 24, 48 and 72 h tumbling periods, vials were withdrawn and centrifuged at 3500 rpm and the concentration of HAdV in the supernatant was measured by qPCR accordingly. For the desorption experiments, equilibrium-adsorbed solids was prepared in the same way.

5.2.5. Optimal solid/liquid ratio determination

Based on MLSS data from Section 5.2.2, sludge samples were diluted with DI water pH = 7) to 9 mL in glass tubes with the S/L ratio of 1:4000, 1:20000 and 1:40000 as dry weight. Then 1 mL of virus solutions with HAdV concentration of $\sim 10^{10}$ and $\sim 10^6$ were added to diluted sludge. The tubes were place on a tumbler and rotated at 20 rpm for 72 h. The sludge-virus solution was centrifuged at 3500 rpm and the concentration of HAdV in the supernatant was measured by qPCR accordingly.

5.2.6. HAdV adsorption

The secondary sludge samples were well-mixed and settled for 1 h. The supernatant was filtered through 0.22 µm syringe-driven PVDF filter units, and the pH was then adjusted to 7. Sludge samples were diluted with DI water (pH = 7) and the sludge filtrate respectively to 9 mL in glass tubes with the S/L ratio of 1:10000 and 1:20000 as dry weight. Then 1 mL of virus solutions with HAdV concentration of $\sim 10^{10}$ and $\sim 10^6$ were added to diluted sludge. The samples were tumbled at 20 rpm until the equilibrium was reached (determined in Section 5.2.4), and then centrifuged at 3500 rpm for 10 min. HAdV concentration in the supernatant was quantified using qPCR.
5.2.7. Sorption isotherm experiments

Human adenovirus stock was diluted with DI water to series of desired concentration (10^6 - 10^{10} virus/ml) in 10 mL tubes. Based on the MLSS concentration measured Section 5.2.2, proper amount of well-mixed sludge was added to virus suspension, so the S/L ratio of the solution matched the optimal ratio determined in Section 5.2.5. Tubes were mixed on a tumbler at 20 rpm for 48 h. After the equilibrium period was reached, vials were centrifuged at 3500 rpm and then the supernatant was collected to measurement assay. For each concentration of virus, a control tube without sludge was made in order to monitor the loss of virus due to inactivation and sorption to the tubes. Control tubes were treated the way as the experimental tubes. This experiment was implemented in duplicate.

The virus concentration on solids was determined according to mass balance:

\[
C_S = \frac{(C_I - C_L)}{M} \quad [1]
\]

Where, \(C_I\), \(C_L\), and \(C_S\) are the virus concentration in liquid phase of control (virus/mL), in the experimental liquid phase (virus/mL), and sorbed to the solid (virus/g), respectively, and \(M\) is the total mass of solid per unit volume of virus suspension (g/mL) in each experimental tube. Data from sorption experiment were then illustrated by Freundlich equation:

\[
\log C_S = \log K_F + n \log C_L \quad [2]
\]

Where, \(K_F\) is the Freundlich constant, which can be used to estimate the adsorption capacity of sorbent; \(n\) is the slope of the curve, which is related to the adsorption intensity (Voice and Weber, 1983).

5.2.8. Sequential desorption experiments

Desorption experiment was implemented as follow: experimental tubes with ~10^6
virus and optimal S/L ratio were made as determined in Section 5.2.5. After reaching equilibrium, the supernatant was removed after centrifuge at 3500 rpm, and replaced with DI water. Then the tubes were placed on tumbler and rotated at 20 rpm until equilibrium (determined in Section 5.2.4). The procedure was repeated 10 times. The experiment was carried out in duplicate, and control tubes were made accordingly.

5.3. Results and Discussion

5.3.1. Equilibrium time and optimal S/L ratio

Virus concentration in supernatant was 5.09 logs, 4.68 logs, 4.96 logs after 24 h, 48 h and 72 h tumbling. For desorption, virus concentration in the supernatant was 4.29 logs and 4.37 logs after 12 h and 24 h tumbling. As a result, 48 h and 12 h were selected as equilibrium time for adsorption and desorption experiments, respectively. Arraj et al. (2005) observed the different sorption behavior of five types of viruses in mixed liquor of activated sludge, and in most cases of their experiments, it took 48 hours for virus concentration to reach constant in the aeration tank. Comparatively, it only took 45 minutes for coxsackie A9 virus concentration reducing by > 99% in activated sludge. Malina et al. (1975) reported that the decrease of poliovirus in activate sludge supernatant became insignificant 1 hour after initial spiking. It has been suggested that properties of viruses and sorbents are the key factor governing virus sorption behavior (Gerba et al. 1980; Goyal and Gerba 1979; Chattopadhyay et al. 2002; Vilker 1981).

As shown in Table 5-4, when S/L ratio = 1:4000, virus concentration was 2.72 logs and 7.46 logs, both of which were more than 1 logs lower than the control. In contrast, 0.69 log and 0.59 log of HAdV was lost due to adsorption at the ratio 1:20000. Further dilution to the ratio of 1:40000 led to negligible adsorption. In this study, S/L ratio of 1:20000 was applied in all experiments.
5.3.2. HAdV adsorption to sludge using DI water and sludge filtrate as solute

As shown in Table 5-5, higher solid content led to greater virus adsorption. This is because more adsorption sites are available for viruses to attach. Previous studies have demonstrated that organic substances, such as proteinaceous matter generally inhibit virus adsorption by competing sorption sites with viruses (Oza and Chaudhuri 1977; Bales et al. 1993; Lo and Sproul 1977; Stagg et al. 1977). Pieper et al. (1997) observed sewage-derived organic matter decreased adsorption of PRD 1 coliphage to aquifer gains. Bradford et al. (2006) found that presence of manure retarded the adsorption of MS-2 and φX174 to sand. However, our data clearly shows that virus adsorption to sludge particles was stronger in sludge filtrate (DOC = 8.1 mg/L) compared to DI water, as less HAdV was present in the liquid phase of the mixture. The results suggest that the effect of some other components in activated sludge, presumably inorganic ions such as Ca$^{2+}$ and Mg$^{2+}$, supersedes the effect of organic matter, resulted in a net enhancement in virus adsorption. Both HAdV and activated sludge particles are negatively charge at neutral pH in aqueous environment (Liao et al. 2000; Steiner et al. 1976; Michen and Graule 2010), and it causes a repulsion force between each other. The shielding effect is more profound at higher ionic strength, which prevents virus and sludge particles from interacting with each other. The electrostatic repulsion between particles is weakened, and thus adsorption is strengthened. Furthermore, according to DLVO theory the electric double layer around viruses and sorbents is suppressed because of high ionic strength. Virus and sludge particles have greater chance to get close to each other, and thus adsorption is increased. The composition of activated sludge is complex and could be prominently varied. Multivalent ions such as Ca$^{2+}$ are much more effective to alter virus sorption compared to monovalent, such as Na$^{+}$ (Bales et al. 1991; Redman et al. 1999; Lance and Gerba 1984). The effect of organic matter on virus sorption is dependent on its
properties (Zhuang and Jin 2003b). More research is needed to understand the contribution of each component in wastewater to virus adsorption. Mathematic models may be built to describe the virus adsorption as a function of ionic strength and the concentration of organic matter.

5.3.3. Adsorption isotherm of HAdV

Sorption isotherm curves of mixed liquor sludge and primary sludge are plotted in Figure 5-1. The $K_F$ values for the two types of sludge were $3.66 \times 10^4$ and $3.92 \times 10^4$, while $n$ values were 1.04 and 1.01, respectively. No significant difference was found between the two types of sludge when t test was applied on $K_F$ and $n$ values. The results indicate that these two types of sludge particles exhibited similar sorption capacity and intensity despite the fact that the organic concentration in samples with primary sludge (DOC = 2.9 mg/L) was much higher compared to samples with secondary sludge (DOC = 0.15 mg/L). The effect of organic matter might be offset by other components in wastewater water, such as ions, as described above.

Wong et al. (2013) established isotherms for soils with 2% and 8% organic content, in which the $K_F$ values were $2.2 \times 10^3$ and $5.0 \times 10^2$, while the $n$ values were 1.04 and 1.07, respectively. Comparing to our data, it suggests that sludge in wastewater treatment process may have a higher capacity, but similar intensity to adsorb adenovirus comparing to soils. Clarke et al. (1961) developed an isotherm for poliovirus (type I) in activated sludge with the parameters $K_F = 7.4 \times 10^2$, and $n = 1.02$ (isotherm curve was re-plotted based on the readings from the original graph). Vilker et al. (1980) also conducted sorption experiments on poliovirus concentration in activated sludge and obtained similar parameters ($K_F = 7.4 \times 10^2$, $n = 1$), based on their isotherm curve $C_S = 0.63M^{-0.81}C_L$. It suggests that poliovirus has less affinity to sludge
particles comparing to adenovirus as the $K_F$ value of poliovirus is lower. Farrah et al. (1978) reported poliovirus showed higher adsorptive capacity than rotavirus in activated sludge, but their sorption experiment only included 5 min mixing of sludge floc and virus for adsorption.

Freundlich isotherms have been widely used in other aspects related to virus sorption. Bitton et al. (1976) used Freundlich isotherm to describe sorption behavior of poliovirus to magnetite in water and wastewater, and they found virus adsorption was affect by cations, but not by variation of pH from 5 to 9. Burge and Enkiri (1978) applied on bacteriophage $\phi X$-174 with 5 types of soils. Decent accordance to Freundlich isotherm was observed in 4 types of soils, while higher content of organic matter that might block adsorption was attributed to the poor correlation in the other soil. With the assistance of Freundlich isotherm, Moore et al. (1981) reported that poliovirus adsorption to soils and minerals was negatively correlated with organic content and negative surface charge on the substrates.

5.3.4. HAdV desorption from sludge particles

Figure 5-2 shows the percentage of HAdV desorbed from each sequential desorption experiment. In both primary and secondary sludge, around 10% of HAdV was detached from sludge particles, and then the rate became slower. The cumulative percent over the 4 sequential desorption experiment was 23.8% and 16.9%, respectively. Statistical analysis showed no significant difference ($p > 0.05$) of HAdV desorption between the two types of sludge. Virus concentration in the liquid phase was below detention limit after the 5th sequential experiment. Our data suggests that desorption of HAdV from sludge particles was not very significant, and it is consistent to previous studies: Clarke et al. (1961) found only a small fraction
adsorbed poliovirus detached from sludge particles and then suggested the sludge-virus matrix was stable. Pepper et al. (2006) reported less than 8% of indigenous coliphage was washed out from biosolids-soil matrix. Bitton et al. (1984) suggested sludge-soil matrix showed strong capacity to retain enteroviruses. Hurst and Brashear (1987) also reported similar results that no prominent desorption of viruses from sludge after land application. In the future, desorption isotherms of viruses need to be built to further evaluate the reversibility of virus adsorption to sludge particles.

5.4. Implications

In this study, we found adsorption of human adenovirus in primary and secondary sludge was well accordant to Freundlich isotherms. The two types of sludge demonstrated very similar behavior of adsorbing human adenovirus. Overall, virus desorption from sludge particles was insignificant. More HAdV was desorbed from primary sludge than from secondary sludge, but the difference was not statistically significant. Greater adsorption of HAdV was observed when liquid phase of activated sludge was used as solute compared to DI water, and it might be a result of compound effect of the inorganic ions (enhance virus adsorption) and organic substances (inhibit virus adsorption).

Although removal of viruses by activated sludge has been frequently reported, mechanisms of virus adsorption to sludge particles and the role of sludge components are still unclear. More studies are needed to further evaluate the fate and transport dynamics of viruses in wastewater systems. Sorption and desorption isotherms in dewater sludge/biosolids should be established since dewatered sludge will be transferred from wastewater to land application. Virus transport to the water
environment is likely to be governed by desorption. Complexity and diversity of wastewater properties is the major obstacle to predict virus transport. The impact of each wastewater component on virus adsorption and desorption should be isolated and link to virus surface properties, such as morphology, hydrophobicity, and isoelectric point.
Table 5-1. Virus partitioning/removal due to sorption in activated sludge

<table>
<thead>
<tr>
<th>Virus</th>
<th>Solids type</th>
<th>Virus partitioning/removal due to sorption</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coxsackie virus</td>
<td>Activated sludge</td>
<td>99.99% removal after 6 h</td>
<td>Clarke et al. 1961</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Activated sludge</td>
<td>67% - 99.8% on solids depending on virus type</td>
<td>Gerba et al. 1980</td>
</tr>
<tr>
<td>Echovirus</td>
<td>Activated sludge</td>
<td>21% - 45% on solids depending on solid concentration</td>
<td>Vilker and Kamdar 1980</td>
</tr>
<tr>
<td>Coxsackievirus</td>
<td>Activated sludge</td>
<td>0.8% - 22% removal</td>
<td>Zheng and Liu 2007</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Activated sludge</td>
<td>0% - 99.6% on solids depending on virus type</td>
<td>Arraj et al. 2005</td>
</tr>
<tr>
<td>T4, f2</td>
<td>Activated sludge</td>
<td>~ 85% on solids</td>
<td>Balluz et al. 1977</td>
</tr>
<tr>
<td>Hepatitis A virus, poliovirus, rotavirus, MS2,φX174</td>
<td>Activated sludge</td>
<td>Distribution of virus in solid and liquid phase: 18:82</td>
<td>Balluz et al. 1978</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Activated sludge</td>
<td>68.4% - 98.4% adsorbed on solids depending on virus type</td>
<td>Farrah et al. 1978</td>
</tr>
<tr>
<td>f2 coliphage</td>
<td>Activated sludge</td>
<td>&gt; 99% on solids</td>
<td>Malina et al. 1975</td>
</tr>
<tr>
<td>Poliovirus, rotavirus</td>
<td>Activated sludge</td>
<td>55% on solids</td>
<td></td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Raw sewage</td>
<td>42% on solids</td>
<td></td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Primary sludge</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Secondary sludge</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(aeration chambers)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Final effluent</td>
<td>92% on solids</td>
<td>Rao et al. 1987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88% on solids</td>
<td></td>
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<td>Rotavirus</td>
<td>Secondary sludge</td>
<td>83% - 99% adsorbed on solids</td>
<td>Moore et al. 1978</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Secondary sludge</td>
<td>Virus concentration in the settled sludge is 3 – 4 logs higher compared to the filtered sludge supernatant</td>
<td>Simmons et al. 2011</td>
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<tr>
<td>Human adenovirus, human enterovirus, norovirus</td>
<td>Secondary sludge (membrane tank)</td>
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</table>

180
<table>
<thead>
<tr>
<th>Virus</th>
<th>Solids type</th>
<th>Virus desorption</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus</td>
<td>Activated sludge</td>
<td>A small fraction of virus desorbed from sludge</td>
<td>Clarke et al. (1961)</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Sludge after land application</td>
<td>No significant desorption</td>
<td>Hurst and Brashear (1987)</td>
</tr>
<tr>
<td>Coliphage</td>
<td>Biosolids soil matrix</td>
<td>Less than 8%</td>
<td>Pepper et al. (2006)</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>Sludge-soil matrix</td>
<td>No significant desorption</td>
<td>Bitton et al. (1984)</td>
</tr>
</tbody>
</table>
Table 5-3. TSS and DOC in primary and secondary sludge

<table>
<thead>
<tr>
<th></th>
<th>TSS, g/L</th>
<th>DOC, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary sludge</td>
<td>0.71</td>
<td>40.6</td>
</tr>
<tr>
<td>(diluted by 30 times)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary sludge</td>
<td>2.66</td>
<td>8.10</td>
</tr>
</tbody>
</table>
Table 5-4. Virus concentration in supernatant with different S/L ratio

<table>
<thead>
<tr>
<th>Original virus conc.</th>
<th>~ 5 logs</th>
<th>~ 9 logs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.31 logs</td>
<td>8.77 logs</td>
</tr>
<tr>
<td>S/L = 1:4000</td>
<td>2.72 logs</td>
<td>7.46 logs</td>
</tr>
<tr>
<td>S/L = 1:20000</td>
<td>3.62 logs</td>
<td>8.18 logs</td>
</tr>
<tr>
<td>S/L = 1:40000</td>
<td>4.38 logs</td>
<td>8.65 logs</td>
</tr>
</tbody>
</table>
Table 5-5. Comparison of DI water and sludge filtrate as solute for HAdV adsorption#

<table>
<thead>
<tr>
<th>Original virus conc.</th>
<th>~ 9 logs</th>
<th>~ 5 logs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/L = 1:10000</td>
<td>7.21 logs</td>
<td>6.40 logs</td>
</tr>
<tr>
<td>S/L = 1:20000</td>
<td>8.65 logs</td>
<td>8.27 logs</td>
</tr>
</tbody>
</table>

#Virus concentration in the table represents the log virus concentration in the liquid phase
*BDL: below detection limit
Figure 5-1. Adsorption isotherm curves (1) primary sludge; (2) secondary sludge*

*Filled and hollow cycles are replicates
Figure 5-2. Percentage of HAdV desorbed from sludge particles in sequential experiments: (1) Primary sludge; (2) Secondary sludge
REFERENCES


Boche, R. D., & Quilligan, J. J. (1966). Adsorption to Glass and Specific Antibody Inhibition of Iodine125 Labeled Influenza Virus. The Journal of Immunology, 97(6), 942-950.


Comparison between adsorption of poliovirus and rotavirus by aluminum hydroxide and activated sludge flocs. *Applied Environmental Microbiology, 35*(2), 360-363.


National Research Council (NRC). (2002). Biosolids applied to land, National Academies, Washington, DC.


Redman, J. A., Grant, S. B., Olson, T. M., Adkins, J. M., Jackson, J. L., Castillo, M. S.,


from exposure to tropical coastal waters impacted by terrestrial dry-weather runoff. *Environmental Science and Technology, 45*(17), 7158-7165.


