Fate of Viruses in Water Systems

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Fate of Viruses in Water Systems

Irene Xagoraraki[1]; Ziqiang Yin[2]; and Zhassulan Svambayev[3]

Abstract: This paper reviews the state of knowledge regarding human viruses in water systems from an environmental engineer's perspective. The authors describe (1) viruses of concern and potential human diseases; (2) waterborne outbreaks related to viruses; (3) the sources, reservoirs, and fate of viruses in the environment; (4) the use of viruses as microbial source tracking tools; (5) virus survival and virus transport; (6) virus concentration and detection methods; (7) the fate of viruses in water treatment; (8) the removal of viruses in full-scale, bench, and pilot-scale conventional and membrane bioreactor (MBR) wastewater systems; and (9) other issues related to viruses in water systems such as the role of bacterial viruses (phages) and viral risk assessment. 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. 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. Overall, virus presence in water and wastewater is a difficult problem for environmental engineers because of prevalence, infectivity, and resistance of viruses to disinfection. Environmental engineers should be aware that even state-of-the-art wastewater treatment plants may not be able to eliminate viruses from wastewater, and viruses potentially escaping from drinking water treatment plants because of technical and management deficiencies may lead to human exposure and disease. The knowledge and tools summarized in this paper provide basic information needed to make decisions for efficient water and wastewater management and reduction of risk of human exposure.
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Viruses of Concern in the United States

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 (Tani 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. 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 EPA's contaminant candidate lists (CCL) (Table 1).

Table 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 2,618 species (ICTV 2012). Another system is the 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. 2003b). 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. 2003b; 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

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Table 1. Human Viruses in the Environmental Protection Agency Contaminant Candidate Lists (CCL)

Virus	Family	Classification	CCL 1	CCL 2	CCL 3
Adenoviruses	*Adenoviridae*	Group I (double strand DNA)	Yes	Yes	Yes
Enteroviruses[a]	*Picornaviridae*	Group IV (positive single-stranded RNA)	—	—	Yes
Coxsackieviruses	*Picornaviridae*	Group IV (positive single-stranded RNA)	Yes	Yes	—
Echoviruses	*Picornaviridae*	Group IV (positive single-stranded RNA)	Yes	Yes	—
Hepatitis A viruses	*Picornaviridae*	Group IV (positive single-stranded RNA)	—	—	Yes
Caliciviruses	*Caliciviridae*	Group IV (positive single-stranded RNA)	Yes	Yes	Yes

Note: DNA = deoxyribonucleic acid; RNA = ribonucleic acid.
[a]Polioviruses, coxsackieviruses, and echoviruses are generally referred to as enteroviruses.

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 (HAV) 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, whereas the associated disease in humans is mainly gastroenteritis (Farkas et al. 2008). Noroviruses are the most common etiologic agents in the *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 because 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).

Waterborne Outbreaks Related to Viruses

It has been estimated that 2–12 million people die per year from waterborne diseases (Gleick 2002; WHO 2011). Most of the waterborne outbreaks in the United States have been related to microbial agents (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 EPA 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, whereas 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 Centers for Disease Control and Prevention (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 underreported and it is difficult to specify the agents (Mena 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 since 1980 (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; 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). 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 nonendemic area for hepatitis E (Favorov et al. 1992, 1999; Aggarwal and Krawczynski 2000), and outbreaks due to hepatitis E have not 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 United States (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).

Sources and Fates of Viruses in the Environment

Sources of Viruses in the Environment

The sources and reservoirs of human viruses are shown in Fig. 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 7,000 infectious viruses per liter

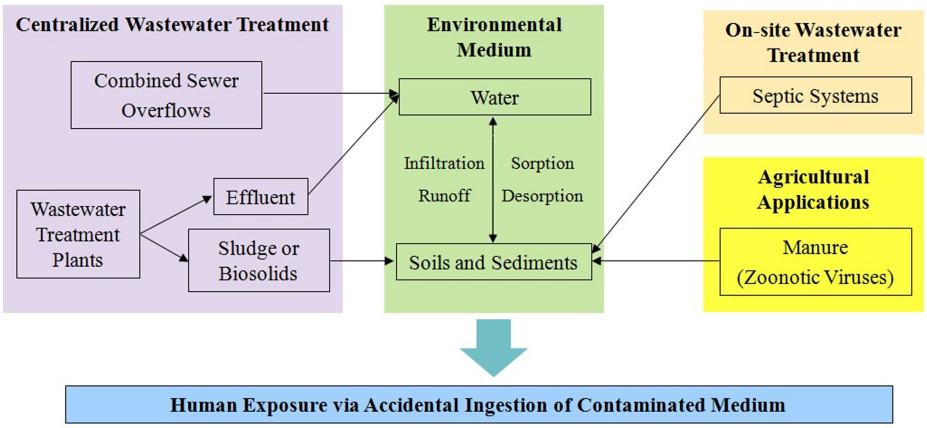

Fig. 1. Sources of viruses in the environment

(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. 2008).

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).

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 microbial source tracking (MST) tools is lack of host specificity (Ahmed et al. 2008; Gordon 2001). 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, 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-specific ribonucleic acid (FRNA) phage (Lee et al. 2009; Smith 2006; Stewart-Pullaro 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. To make viruses detectable and to efficiently use them as MST tools, a concentration procedure is usually required involving filtration of large amounts of water during sampling.

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. 2009b; Xagoraraki et al. 2007).

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 resuspend into the water column because adsorption may be reversible. Resuspension 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. 2003a; Lieberman et al. 1994; 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. (2003a) 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. 1994) in which 30 public water supply wells were examined, the authors reported that 24% of the samples were positive for culturable viruses. Also, the U.S. 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 ground water 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 because of water 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).

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 ultraviolet (UV), and presence of microbiological flora (Bosch et al. 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 et al. 1989). In groundwater, the presence of indigenous microorganisms is the important feature in inactivation of enteroviruses (Gordon and Toze 2003). Exposure to UV light or sunlight can enhance virus inactivation in the environment. For example, to achieve an inactivation rate of 99% for poliovirus without UV light in marine water, 52 days were needed, whereas in the presence of sunlight, only 21 days were required (Rzezutka and Cook 2004).

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 of 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; and $1/n$ 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 (1) clayey soils have higher virus sorption capacity, (2) an increase in cation concentration in solution can increase virus sorption, and (3) pH affects virus sorption. The presence of organic matter (OM) enhances virus transport (Bixby and O'Brien 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 IEP and hydrophobicity, play major roles in controlling virus sorption and transport. The size and IEP of selected viruses are summarized in Table 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 60 nm 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, whereas 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 $NaHCO_3$-NaCl solution (pH = 7), the zeta potential of poliovirus is -1.8 ± 0.3 and -5.9 ± 0.9 mV at ionic strengths of 0.3 and 0.2 M, respectively (Murray and Parks 1980). At low zeta potentials, viruses tend to coagulate or flocculate.

Table 2. Summary of Virus Surface Properties Affecting Sorptive Removal from Water

Virus[a]	Virion size (nm)	IEP	References
Enterovirus	22–30	4.0–6.4	Minor (1987), Grce and Pavelić (2005), Murray and Parks (1980), Butler et al. (1985), Zerda and Gerba (1984)
Coxsackieviruses		4.75–6.75	
Echoviruses		4.0–6.4	
Hepatitis A viruses	27–28	2.8	Minor (1987, Nasser et al. (1992)
Caliciviruses	30–40	5.5–6.0[b]	Carter and Madeley (1987), Goodridge et al. (2004)
Adenoviruses	70–140	3.5–4.5	Nermut (1987), Trilisky and Lenhoff (2007), Wong et al. (2012b), Stewart et al. (1991)

[a]All viruses in CCL are nonenveloped and icosahedral in shape.
[b]For Norwalk virus (a member of noroviruses).

It has been suggested that viruses with a lipid envelope are generally hydrophobic, whereas 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 (MS2) 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 et al. 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 (25 ml sample volume with 2% biosolids) from solid media, 10^3 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.

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 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 deoxyribonucleic acid (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 (RT-PCR) is used to produce a complementary strand (cDNA) for ribonucleic acid (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 (qPCR) 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 Fig. 2. Sample collection and pretreatment is a critical aspect of all environmental virology methods and pretreatment methods are also shown in Fig. 2. Virus concentration in natural water bodies is usually low, and preconcentration of viruses is often the most important step for effective detection.

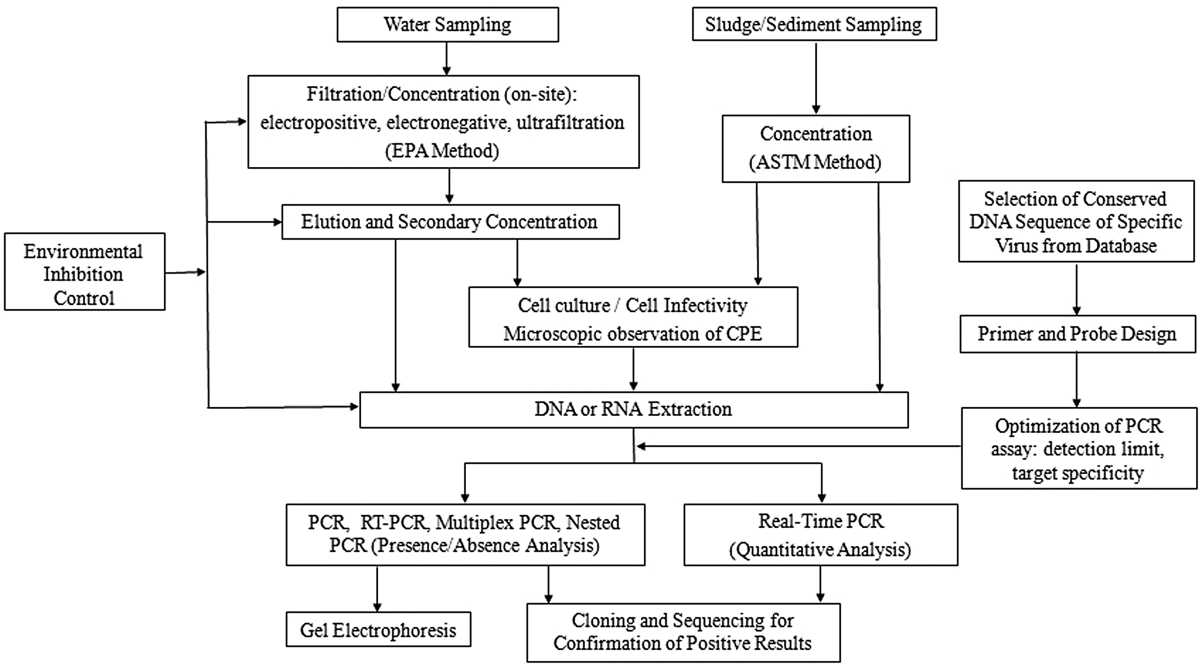

Fig. 2. Summary of virus elution and detection methods

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 pretreatment. 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. Eluates containing viruses are flocculated by lowering pH. Flocs are isolated by centrifugation and resuspended 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 (ASTM 2002). 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. The pH is neutralized before eluted samples are passed through membrane filters.

Fate of Viruses during Water Treatment

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). In general, even though most water treatment plants can achieve more than 4 log virus reduction (Payment and Franco 1993, Payment et al. 1985), viruses have been detected in finished water on several occasions. 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 min contact time is generally needed compared with 5 min 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% of enteric viruses were removed during the dry season, whereas 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 which thereby protected the viruses from final chlorination.

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. (2005a) 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 with 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 1999a). 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 1999a, c). 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 0.4 to 1.24 mg/L (Katzenelson et al. 1979), whereas adenovirus removal slightly increased as a consequence of longer contact time (Thurston-Enriquez et al. 2005b). 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, 2009). Higher UV dose can steadily increase inactivation of a variety of viruses such as echovirus, coxsackievirus, poliovirus, and adenovirus (Gerba et al. 2002a; Ko et al. 2005; Thompson et al. 2003; Simonet and Gantzer 2006). Some viruses cannot 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. 2002a; Ko et al. 2005; Nwachuku et al. 2005; Thurston-Enriquez et al. 2003b).

Fate of Viruses in Wastewater Treatment Systems

Virus Removal in Full-Scale Wastewater Utilities

Wastewater is a primary source of human viruses in the environment. Conventional full-scale wastewater treatment utilities release infectious and noninfectious viruses in their effluent (Katayama et al. 2008; Haramoto et al. 2007; Hewitt et al. 2011; Petrinca et al. 2009; Aulicino et al. 1996; Costan-Longares et al. 2008;

Table 3. Virus Removal in Full-Scale Membrane Bioreactors

Membrane pore size (μm)	Virus (source)	Detection methods	Removal efficiency (logs)	Reference
0.4	F-specific coliphage	Plaque assay	6.0	Zanetti et al. (2010)
0.4	Somatic coliphage	Plaque assay	4.0	Zanetti et al. (2010)
0.1	HAdV	qPCR	4.1–5.6	Kuo et al. (2010)
0.4	Norovirus I	qPCR	0–5.3[a]	da Silva et al. (2007)
	Norovirus II		0–5.5[a]	
NA	HAdV	qPCR	3.4–4.5[a]	Simmons and Xagoraraki (2011)
	Enterovirus		2.9–4.6[a]	
0.1	HAdV	qPCR	4.1–6.3	Simmons et al. (2011)
	Enterovirus		4.1–6.8	
	Norovirus (II)		3.5–4.8	

Note: NA = not applicable.
[a]Obtained from graphs.

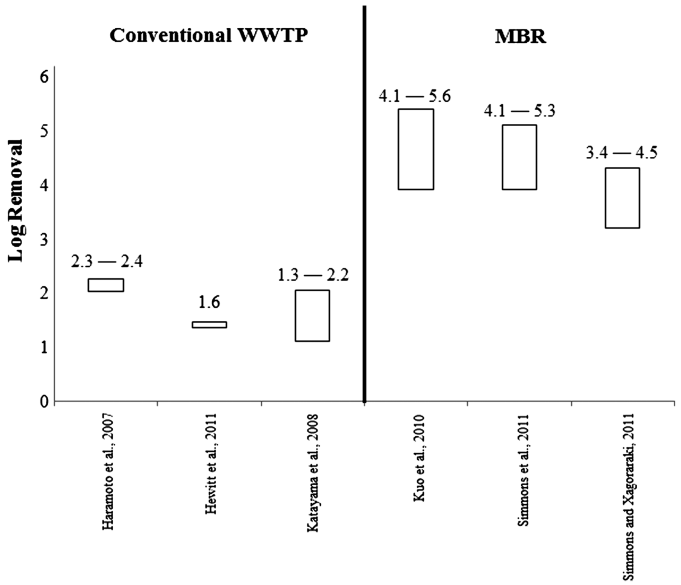

Fig. 3. Adenovirus removal in full-scale wastewater treatment plants

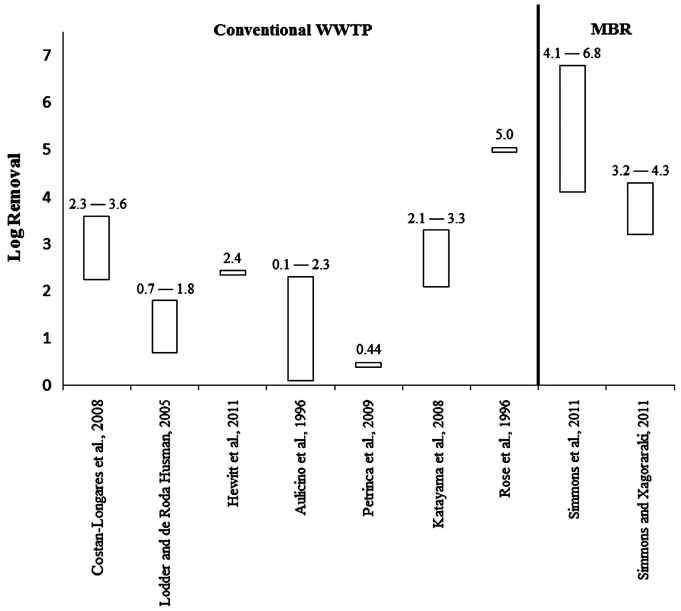

Fig. 4. Enterovirus removal in full-scale wastewater treatment plants

Lodder and de Roda Husman 2005; Rose et al. 1996; Nordgren et al. 2009; Haramoto et al. 2007; Kitajima et al. 2009; Payment et al. 2001; Prado 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 3). For example, Kuo et al. (2010) reported 4.1–5.6 log removals for human adenoviruses, whereas 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 Figs. 3–6. Overall, full-scale MBR plants achieved higher virus removals. Adenovirus removal in WWTPs prior to disinfection (Table 3 and Fig. 3) ranged from 1.6 to 2.4 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 to 6.3 logs (Kuo et al. 2010; Simmons et al. 2011; Simmons and Xagoraraki 2011).

Fig. 4 shows a summary of enterovirus removals in full-scale WWTPs. In conventionally treated wastewater prior to disinfection, virus removals ranged from 0.7 to 2.9 logs (Lodder and de Roda Husman 2005; Costan-Longares et al. 2008; Hewitt et al. 2011). Conventional plants with disinfection produced higher virus removals: up to 5.23 logs (Aulicino et al. 1996, Petrinca et al. 2009; Katayama et al. 2008; Costan-Longares et al. 2008; Rose et al. 1996). MBR plants without disinfection removed enteroviruses from 4.1 to 6.8 logs (Simmons et al. 2011).

As shown in Fig. 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 removals from 1.0 to 2.7 logs (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 Fig. 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 to 4.9 logs were observed (da Silva et al. 2007; Simmons et al. 2011).

Virus Removals in Bench and Pilot-Scale MBR Systems

Bench and pilot-scale MBR studies have been performed to describe virus removal. MS2 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 (20–25 nm),

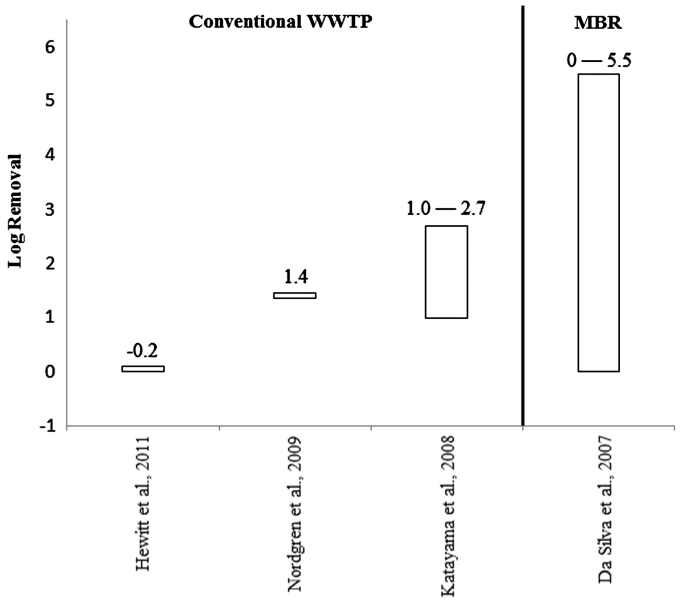

Fig. 5. Norovirus I removal in full-scale wastewater treatment plants

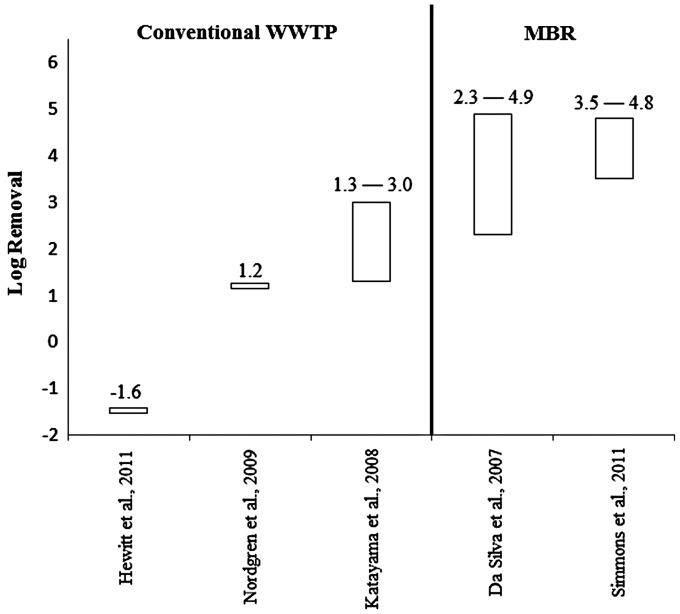

Fig. 6. Norovirus II removal in full-scale wastewater treatment plants

and low IEP (3.9) (Zerda et al. 1985) 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 and Leiknes 2006) and thus make MS2 a good indicator and surrogate for virus studies with membrane systems (Shang et al. 2005; Comerton et al. 2005). Both indigenous and lab-cultured MS2 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 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 because of 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 transmembrane 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 (Fig. 7). Madaeni et al. (1995) reported that hydrophobic polyvinylidene fluoride (PVDF) membrane (pore size $= 0.22$ μm) could remove about 99% of poliovirus, whereas 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 MS2 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 and 0.22 μm pore sizes, whereas Lv et al. (2006) indicated that a 0.1 μm membrane was more effective than a comparable 0.22 μm membrane. Fiksdal and Leiknes (2006) reported that phages were poorly removed during MBR treatment without precoagulation/flocculation, even using ultrafiltration membranes.

Viruses in Biosolids

Most wastewater virus studies report numbers of viruses in effluent or removal efficiencies that reflect virus concentrations in influent and effluent. Because 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 United States, approximately 5.6 million dry tons of biosolids are generated annually, 60% of which are land applied as a soil amendment (NRC 2002). The U.S. EPA divides biosolids into two classes: (1) class A or pathogen-free biosolids and (2) 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 pathogens 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 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).

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 and Kushmaro 2011; Rosenberg et al. 2010; Wu and Liu 2009; Otawa et al. 2007). In activated sludge, the

Table 4. Virus Removal in Bench and Pilot-Scale Membrane Bioreactors

Scale	Membrane pore size	Virus (source)	Removal efficiency[a] (logs)	Reference
Bench	0.4 μm	MS-2	0.4–2.5	Shang et al. (2005)
	0.2 μm	MS-2	Average 6.7	Fiksdal and Leiknes (2006)
	0.45 μm	MS-2	0.31–1.5	Oh et al. (2007)
	UF and NF	MS-2	2 for UF, 4 for NF	Hu et al. (2003)
	0.1 and 0.22 μm	T4 coliphage	5–8 for 0.1 μm, 3.5–6 for 0.22 μm	Lv et al. (2006)
	0.1 and 0.22 μm	T4 coliphage	5.5	Zheng and Liu (2007)
	0.1 and 0.22 μm	T4 coliphage	6	Zheng et al. (2005)
	0.4 μm	Somatic coliphage	1.5–2.5	Wu et al. (2010)
Pilot	300 kDa	MS-2	No plaques observed	Cicek et al. (1998)
	0.04–0.1 μm	MS-2	1.0–4.4	Hirani et al. (2010)
	0.2 μm	MS-2	3.8	Ravindran et al. (2009)
	0.1 μm	Somatic coliphage	No plaques observed	Ahn et al. (2001)
	0.03 μm	Somatic coliphage	3.7	Wong et al. (2009a, b)
	0.4 μm	F-specific coliphage	>4.0	Tam et al. (2007)
	0.1 μm	F-specific coliphage	No plaques observed	Ahn et al. (2001)
	0.04 μm	Enteric cytopathogenic bovine orphan virus	Not detectable in effluent	Krauth and Staab (1993)
	0.45 μm	Norovirus enterovirus	$-0.19to-0.01$, $-0.05to-0.03$	Ottoson et al. (2006)

[a]Removal efficiencies were calculated based on results from plaque assays.

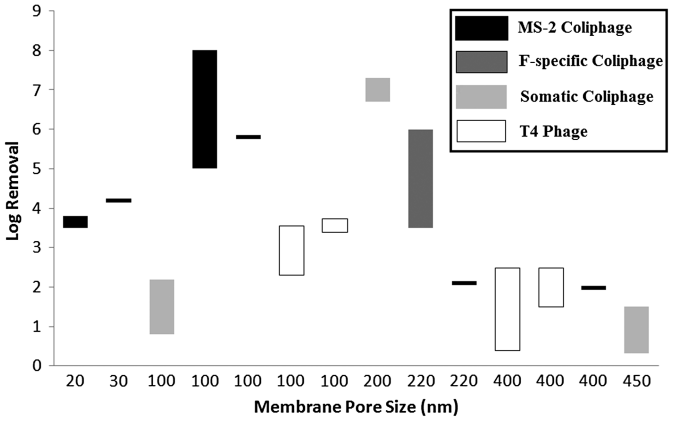

Fig. 7. Virus removal as a function of membrane pore size in bench and pilot scale MBR systems

Table 5. Virus Occurrence in Dewatered Sludge and Class B Biosolids

Author	Detection method	Viruses	Occurrence average
		Dewatered sludge	
Bofill-Mas et al. (2006)	qPCR	Adenoviruses	1.1×10^2 copies/g
Monpoeho et al. (2001)	RT-PCR	Enteroviruses	4.8×10^4 copies/10 g
	Cell culture		7 MPNCU/10 g[a]
Viau and Peccia (2009)	qPCR	Adenoviruses	2.5×10^4 copies/g
Wong et al. (2010)	qPCR	Adenoviruses	1.9×10^8 copies/g
		Enteroviruses	2.3×10^5 copies/g
	Cell culture	Adenoviruses	2210 MPN/4 g
		Enteroviruses	
		Class B biosolids	
Bofill-Mas et al. (2006)	qPCR	Adenoviruses	10^3 copies/g
Monpoeho et al. (2001)	RT-PCR	Enteroviruses	1.06×10^4 copies/10 g
	Cell culture		9 MPNCU/10 g
Monpoeho et al. (2004)	RT-PCR	Enteroviruses	1.2×10^4 copies/g
	Cell culture		38.2 MPNCU/g
Viau and Peccia (2009)	qPCR	Adenoviruses	5×10^5 copies/g
Wong et al. (2010)	qPCR	Adenoviruses	7.5×10^5 copies/g
		Enteroviruses	1.9×10^4 copies/g
		Norovirus GI	5×10^4 copies/g
		Norovirus GII	1.5×10^5 copies/g
	Cell culture	Adenoviruses	480 MPN/4 g
		Enteroviruses	
		Norovirus GI	
		Norovirus GII	

[a]MPNCU = most-probable-number cytopathogenic units.

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 (Mazaheri Nezhad Fard et al. 2010; Canchaya et al. 2004; Boyd and Brüssow 2002). Horizontal gene transfer, such as transformation, conjugation and phage mediated transduction, 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 and Schmieger 2001). Very little information is available regarding phage-mediated transduction (Colomer-Lluch et al. 2011; Sander and Schmieger 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 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 biodegradation) 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.

Viral Risk Assessment

Quantitative viral risk assessment (QVRA) studies have been published for wastewater systems. Exposure to human enteric viruses from wastewater-related products (postdisinfected 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 Eq. (1) (van Heerden et al. 2005). The exponential model [Eq. (2)] and beta-Poisson model [Eq. (3)] have been used extensively to represent dose-response relationships (Table 6) and estimate the probability of infection. As α increases, the beta-Poisson model approaches the exponential model (Haas et al. 1999)

$$N = C \times \frac{1}{R} \times I \times 10^{-\mathrm{DR}} \times V \tag{1}$$

$$P_{i/\mathrm{day}} = 1 - \exp(-rN) \tag{2}$$

$$P_{i/\mathrm{day}} = 1 - \left(1 + \frac{N}{\beta}\right)^{-\alpha} \tag{3}$$

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 in water that are capable of infection; and DR is the removal or inactivation efficiency of the treatment process. For recreational water, DR is equal to 0 because no treatment is applied; V is the daily volume of water consumed by individuals; $P_{i/\mathrm{day}}$ is the probability of becoming infected; and α, β and r are dose response parameters (Table 6).

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. 2002b; Eisenberg et al. 2006, 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. 2002b; Eisenberg et al. 2008). For example, during biosolids-based QVRA studies (Gerba et al. 2002b; 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 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. (2002b) 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 from soil ingestion

Table 6. Dose-Response Models for Enteric Viruses

Waterborne virus	Exposure	Dose-response model	Defined parameters	Reference
Enteroviruses 68–71	Ingestion	Beta-Poisson	$a = 0.67$, $\beta = 47.9$	Soller et al. (2004)
Poliovirus 1	Ingestion	Exponential	$r = 0.009102$	Regli et al. (1991), Minor et al. (1981)
Poliovirus 1	Ingestion	Beta-Poisson	$a = 0.1097$, $\beta = 1524$	Regli et al. (1991), Lepow et al. (1962)
Poliovirus 3	Ingestion	Beta-Poisson	$a = 0.409$, $\beta = 0.788$	Regli et al. (1991), Katz and Plotkin (1967)
Coxsackievirus A21	Inhalation	Exponential	$r = 0.0145$	Haas et al. (1999)
Coxsackievirus B4		Exponential	$r = 0.007752$	Haas et al. (1999)
Echovirus 12	Ingestion	Exponential	$r = 0.012771$	Haas et al. (1999)
Echovirus 12	Ingestion	Beta-Poisson	$a = 0.374$, $\beta = 186.69$	Regli et al. (1991), Schiff et al. (1984)
Human adenovirus 4	Inhalation	Exponential	$r = 0.4172$	Haas et al. (1999), Mena and Gerba (2009)
Human caliciviruses	Ingestion	Beta-Poisson	$a = 0.126$–0.5, $\beta = 0.21$–0.84	Soller et al. (2004)
Noroviruses	Ingestion	Exponential	$r = 0.069$	Masago et al. (2006)
Rotavirus	Ingestion	Beta-Poisson	$a = 0.253$, $\beta = 0.422$	Regli et al. (1991), Haas et al. (1999), Ward et al. (1986), Teunis et al. (2008)
Hepatitis A virus	Ingestion	Exponential	$r = 0.548576$	Haas et al. (1999)

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. (2009b) estimated the daily risk of viral infection ranged from 0.2 to 2.4 per 1000 swimmers.

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 because 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 because of the small sizes, prevalence, infectivity, and resistance of viruses to disinfection. In this paper, virus survival and behavior in the environment were briefly described and both virus-associated diseases and their transmission pathways were reviewed. 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 because of 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.

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