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Review

Reducing uncertainty in estimating virus reduction by advanced water treatment processes

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ABSTRACT

Treatment of wastewater for potable reuse requires the reduction of enteric viruses to levels that pose no significant risk to human health. Advanced water treatment trains (e.g., chemical clarification, reverse osmosis, ultrafiltration, advanced oxidation) have been developed to provide reductions of viruses to differing levels of regulatory control depending upon the levels of human exposure and associated health risks. Importance in any assessment is information on the concentration and types of viruses in the untreated wastewater, as well as the degree of removal by each treatment process. However, it is critical that the uncertainty associated with virus concentration and removal or inactivation by wastewater treatment be understood to improve these estimates and identifying research needs. We reviewed the critically literature to assess to identify uncertainty in these estimates. Biological diversity within families and genera of viruses (e.g. enteroviruses, rotaviruses, adenoviruses, reoviruses, noroviruses) and specific virus types (e.g. serotypes or genotypes) creates the greatest uncertainty. These aspects affect the methods for detection and quantification of viruses and anticipated removal efficiency by treatment processes. Approaches to reduce uncertainty may include; 1) inclusion of a virus indicator for assessing efficiency of virus concentration and detection by molecular methods for each sample, 2) use of viruses most resistant to individual treatment processes (e.g. adenoviruses for UV light disinfection and reoviruses for chlorination), 3) data on ratio of virion or genome copies to infectivity in untreated wastewater, and 4) assessment of virus removal at field scale treatment systems to verify laboratory and pilot plant data for virus removal.

Contents

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1. Introduction

Since domestic wastewater will always contain microbial pathogens, it is important that when intended for reuse applications, pathogens must be reduced to levels that do not have an impact on public health. Microbial risk assessments are useful to provide guidance for the needed reductions for treatment process to minimize risks of infection (NRC, 2012). Among the pathogen groups found in wastewater, viruses present the greatest risk because they generally occur in much greater concentrations and have a much greater infectivity (i.e. higher probability of infection with a given exposure), than bacteria and parasitic protozoa. With close to 200 species of enteric viruses, which can occur in wastewater, they represent the greatest number of different species of enteric pathogens (Gerba et al., 2017).

Minimum log reduction values of viruses by treatment trains designed for recycling of wastewater has been suggested. Recycled water intended for irrigation of edible crops requires a 6–7 log reduction (WHO, 2006) and for potable reuse applications (i.e., groundwater recharge and augmentation of surface water supply reservoirs) a 12-log reduction has been suggested (Title 22 and 17 California Code of Regulations. 2015). These reductions are based on assuming infective virus concentrations of 10^5 to 10^6 per liter in raw wastewater based on datasets collected in previous studies (Harwood et al., 2005; Rose et al., 2005). Recent application of molecular methods in wastewater and recycled water settings, suggests that some pathogenic viruses may be occurring in concentrations of upwards of 10^7 to 10^9 genome copies per liter (Gerba et al., 2017; Eftim et al., 2017). However, it is still unknown the relative proportions of infectious to non-infectious virus in these sample types. Viruses in raw sewage are more likely to be infectious due to direct excretion with feces. Moreover, their survival in sewage is facilitated by organic debris of the clinical matrix in which the virus is shed (e.g., feces or vomit) and virus aggregation formation, offering protection in the route to new human hosts (Rusiñol and Girones, 2017).

Several studies have attempted to estimate the impact of risk reduction by different treatment processes for pathogens present in untreated wastewater and at the same time quantifying the risks from viruses (NRC, 2012; Olivieri et al, 2014; WHO, 2017; Soller et al., 2018). However, most have not addressed the uncertainty in these estimates associated with the factors listed in Table 1. Exposure usually presents the greatest amount of uncertainty in risk estimation (Haas et al., 2014). Here we review those factors which exert the greatest influence on uncertainty in risk assessment for viruses in recycled treatment systems.

2. Factors influencing uncertainty in risk assessment for viruses in recycled water

2.1. Estimating virus concentrations in water

Knowing the concentration of infectious viruses in raw sewage entering a treatment facility is critical in assessing the needed efficacy of the entire processes in reducing viruses to acceptable levels. Recent advances in molecular biological methods have revealed that levels of viruses in untreated raw sewage are much greater than previously thought (Gerba et al., 2017). Applications of these advanced molecular based methods to raw sewage indicate that enteric virus levels can reach levels of 9,800,000,000 per liter. It has been documented that some viruses, such as adenoviruses, are much more abundant in wastewater and occur at higher concentration (1000 fold or more) than other common enteric viruses (Kitajima et al., 2014). In addition, real world data on the removal of naturally occurring viruses through wastewater treatment needs further assessment. However, determining the number of infectious viruses in water is challenging because no single method can detect all of the infectious viruses that may be present. Molecular methods, which detect the nucleic acids of viruses, do not inform us as to their infectivity. Methods for determining the infectivity of human viruses depend on documenting their replication in cell culture. Enteroviruses (e.g. poliovirus) were among the first viruses grown in animal cell culture and have been the most studied in water/wastewater. Numerous methods for detecting virus replication in cell culture have been developed (Payment and Trudel, 1993). However, no single cell culture system can be used for all enteric viruses. The propagation of viruses in cell culture followed by detection by the polymerase chain reaction (PCR) assay, termed integrated cell culture (ICC)–PCR, provides a new procedure for monitoring infectious viruses that do not induce cytopathic effect or plaques in cell culture (Reynolds et al., 1996; Chapron et al., 2000). This method also has the advantage of reducing the time for virus detection and increasing detection sensitivity. Unfortunately, only small number of the enteric virus types found in wastewater can replicate in routine cell culture. Even then, different virus types require different cell culture lines and the susceptibility of the cell line to a particular virus may change over time in the laboratory (Payment and Trudel, 1993; Chapron et al., 2000; Condit, 2013). In addition, the cultivation of naturally occurring viruses in wastewater may be less efficient than cultivation of laboratory-adapted strains which have been selected for rapid growth. For example, Ward et al. (1984) found that only one virion of rotavirus in 46,000 in stool resulted in observable growth in cell culture. Adaptation of the virus by two passages in cell culture resulted in a decrease in that ratio to 1:6600. In addition, one virus may mask the presence of other viruses in cell culture because of different growth rates or other factors (Calgua et al., 2002; Carducci et al., 2002). The method selected for assay can also affect the results i.e. suspended cell culture methods usually give a greater number of isolates versus the commonly used monolayer method (Slade et al., 1984). Given the variety of factors influencing viruses known to grow in cell culture the efficiency may range from 0.01% to perhaps 50% (Ikner et al., 2012).

To overcome the limitations encountered with cell culture methods, intercalating dyes such as propidium monoazide (PMA) in conjunction with real time PCR (RT-qPCR or qPCR for RNA or DNA viruses, respectively (PMA-RT-qPCR/qPCR) have been used to determine the potential infectivity of enteric RNA and DNA viruses in water and other environmental matrices (Parshionikar et al., 2010; Karim et al., 2015; Leifels et al., 2015; Fongaro et al., 2016). However, current methods are still limited in this assessment (Rodriguez et al., 2009). Success of such methods depends on knowledge of the mechanism of inactivation of a particular virus and the site of action of a particular disinfectant (Rodriguez et al., 2009; Coudray-Meunier et al., 2013; Gall et al., 2015; Prevost et al., 2016). In addition, complicating this approach is that some viruses such as adenoviruses rendered non-infectious by ultraviolet light can use host cell enzymes to repair DNA damages on their