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Inactivation kinetics of adenovirus serotype 2 with monochloramine

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ABSTRACT

The effect of pH (6–10), temperature (10–30 °C), disinfectant concentration (1–11 mg/l as Cl_2), and ammonia nitrogen-to-chlorine molar ratio (1.3–52) on the inactivation kinetics of adenovirus serotype 2 with monochloramine was investigated by performing batch-reactor experiments with synthetic 0.01 M buffer (phosphate or borate) solutions. The inactivation kinetics was independent of monochloramine concentration and ammonia nitrogen-to-chlorine molar ratio but had strong pH dependence, with the rate of inactivation decreasing with increasing pH. The kinetics at pH 6 and 8 were consistent with pseudo-first-order kinetics, while curves at pH 10 were characterized by a lag phase followed by a pseudo-first-order phase. The rate of inactivation increased with increasing temperature-activation energies of 56.5 kJ/mole (pH 8) and 72.6 kJ/mole (pH 10). The results obtained in this study revealed that monochloramine disinfection might not generally provide adequate control of adenoviruses in drinking water at high pH and low temperature.

1. Introduction

Adenoviruses are non-enveloped, icosahedral-shaped viruses with sizes in the range of 70–100 nm. The adenovirus virion consists of a protein capsid protecting a double-stranded DNA genome of 28–42 kb, and a number of proteins that are associated with the DNA. The capsid is formed by 240 copies of a trimeric hexon protein. There is also a penton complex in each of the 12 icosahedron vertices, each consisting of a pentameric base and a protruding trimeric fiber.

Human adenoviruses include 51 serotypes that are transmitted by the fecal–oral route and through inhalation of aerosols (Jiang, 2006). Adenovirus serotypes 40 and 41 cause enteric diseases, while others affect the respiratory system. Sensitive populations to adenovirus infection include immunocompromised individuals and young children (Jiang, 2006). Adenoviruses have been reported to be present in tap water and treated drinking water (Enriquez et al., 1995; Lee and Kim, 2002; Jiang, 2006), surface water (Pina et al., 1998; Jiang, 2006), coastal seawater (Enriquez et al., 1995; Pina et al., 1998; Fong et al., 2005; Jiang, 2006), treated and untreated wastewater (Enriquez et al., 1995; Pina et al., 1998; Vantarakis and Papapetropoulou, 1999; Jacangelo et al., 2003; Thompson et al., 2003; Jiang, 2006), and swimming pool water (van Heerden et al., 2005; Jiang, 2006). As a result waterborne outbreaks of human adenovirus infection have been reported for both enteric and respiratory adenovirus serotypes (Jiang, 2006; Crabtree et al., 1997).

Free chlorine is currently the most common disinfectant used by US drinking water utilities that treat surface water or

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groundwater under the direct influence of surface water, both for primary disinfection in the treatment plant as well as for providing a disinfectant residual in the distribution system. Although free chlorine is very effective in inactivating adenoviruses, many utilities will be switching to alternative disinfection schemes in order to comply with the recently promulgated Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (US Environmental Protection Agency, 2006a) and Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) (US Environmental Protection Agency, 2006b). An alternative treatment approach under consideration is the use of ultraviolet (UV) light for primary disinfection at the treatment plant followed by combined chlorine for providing a disinfectant residual in the distribution system. UV disinfection can effectively inactivate *Cryptosporidium parvum* oocysts, the primary waterborne pathogen addressed in LT2ESWTR, which in contrast to adenoviruses is highly resistant to free chlorine. Furthermore, compared to free chlorine, UV disinfection does not result in significant formation of disinfection byproducts (DBPs) and combined chlorine forms lower levels of trihalomethanes and haloacetic acids, the two main DBP groups regulated under Stage 2 DBPR.

However, switching from free chlorine to UV/combined chlorine disinfection is resulting in new concerns. Viruses are more resistant to UV inactivation than protozoa (oo)cysts; adenoviruses in particular have been found to be more resistant to UV than any other viral, bacterial and protozoan pathogen of current concern in drinking water (Meng and Gerba, 1996; Shin et al., 2001; Gerba et al., 2002; Jacangelo et al., 2003; Thompson et al., 2003; Thurston-Enriquez et al., 2003; Nwachcuku and Gerba, 2004). Furthermore, information available regarding the inactivation of viruses with combined chlorine is both limited and inconsistent. For example, the inactivation efficiency of adenovirus serotype 2 by monochloramine at pH 8 and 22–23 °C has been reported to be 94% at monochloramine exposure or integrated monochloramine concentration over contact time (CT) of 265 mg × min/l (Ballester and Malley, 2004). In contrast, a higher inactivation efficiency of 99.999% has been reported for the same adenovirus serotype at the lower monochloramine CT of 150 mg × min/l under approximately the same experimental conditions of pH 8 and 25 °C (Baxter et al., 2007). Despite such inconsistency, adenovirus appears to be much more resistant to combined chlorine than to free chlorine. As a result adenovirus and other enteric viruses, which were not a top concern in the past because they are very susceptible to free chlorine inactivation, are now considered an emerging concern. Although specific enteric viruses are not currently regulated, four groups of viruses including adenoviruses are currently included in the Contaminant Candidate List 2 (CCL2) for potential future regulatory development (US Environmental Protection Agency, 2005). A decision on regulatory action will require the availability of a more comprehensive characterization of the inactivation kinetics of adenovirus with monochloramine. Accordingly, the objective of this study is to characterize the inactivation kinetics of adenovirus with monochloramine as a function of pH, temperature, disinfectant concentration, and ammonia nitrogen-to-chlorine molar ratio.

2. Materials and methods

2.1. Host cell preparation and virus propagation

Human lung carcinoma A-549 cells (CCL-185, American Type Culture Collection, Manassas, VA) were suspended in nutrient mixture F-12 Ham Kaighn's modification (Ham's F-12K) medium, containing 10% fetal bovine serum (FBS), 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin-B. This medium is referred to in this study as "complete medium." Cellular monolayers were propagated in this complete medium inside tissue culture flasks at 37 °C inside an incubator fed with humidified air containing 5% CO_2.

Human adenovirus serotype 2 (VR-846, ATCC, Manassas, VA) was used in this study as a model for other adenoviruses due to its simpler culture method and comparable UV resistance (Shin et al., 2001). Three virus shipments (Lots 5, 7, and VP2) were used for the monochloramine disinfection experiments of this study. Viruses from Lots 5 and 7 were used directly without propagation. Six virus stocks (VP2-3–7 and VP2-7AC) were propagated from Lot VP2, according to the following protocol. For all six stocks, viruses were inoculated at a target multiplicity of infection (MOI) of 160–500 plaque-forming units (PFU)/cell onto sub-confluent A-549 cellular monolayers. The medium used for suspending viruses, referred to as "virus medium," was a modified version of the Ham's F-12K medium, containing 2% FBS, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin-B. Inoculated cell monolayers were rocked for 2–3 s at 15-min intervals for 90 min to distribute the virus suspension evenly and to facilitate virus–host cell attachment. Next, the inoculum was removed and complete medium was added to flasks. Infected cellular monolayers were then incubated at 37 °C with humidified air containing 5% CO_2. At 3–4 days post-infection, cellular monolayers had detached from flasks. Some differences should be noted in subsequent steps used for stocks VP2-3–7 and VP2-7AC. For virus stocks VP2-3–7, cells were dislodged using a cell scraper. The suspension in the flasks was freeze-thawed three times. Cell debris and released virus were collected and centrifuged at 230*g* at room temperature for 10 min. The cell-debris pellet was discarded, and the supernatant was filtered through a polyvinylidene fluoride (PVDF) membrane with nominal pore size of 0.45 μm using a vacuum-driven Stericup filter unit (Millipore, Billerica, MA) to remove fine debris and virus aggregates that had not been removed in the centrifugation step. The viruses were aliquoted in 0.01 M phosphate buffer solution (PBS) at pH 8 and the resulting stock stored at −80 °C until used.

Virus stock VP2-7AC was prepared by performing an extra step designed for additional removal of dissolved matter from virus stock VP2-7. A PVDF ultrafiltration (UF) membrane with nominal molecular weight cut-off of 30 kDa (HFM-100; Koch Membrane Systems, Wilmington, MA) installed in an Amicon cell (Millipore, Billerica, MA) was preconditioned by filtering a 3 ml volume of A549 cell debris suspension to clog imperfections in the membrane with the goal of minimizing any subsequent virus losses. A 30 ml volume of virus stock VP2-7