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Interaction of human adenoviruses and coliphages with kaolinite and bentonite

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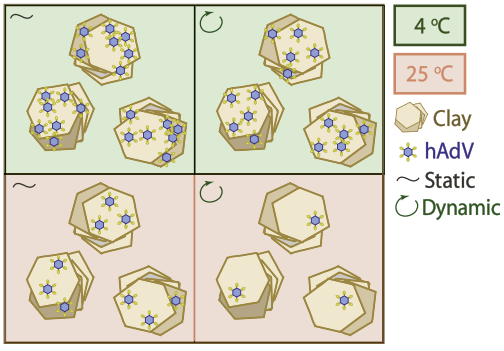
HIGHLIGHTS

- Temperature plays a significant role on virus adsorption onto clays.
- Adenovirus adsorption is higher under static than dynamic conditions.
- For most cases considered, hAdV adsorption is higher at the highest IS.
- The adsorption of both MS2 and ΦX174 is not similar to that of hAdV.

GRAPHICAL ABSTRACT



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ABSTRACT

Human adenoviruses (hAdVs) are pathogenic viruses responsible for public health problems worldwide. They have also been used as viral indicators in environmental systems. Coliphages (e.g., MS2, ΦX174) have also been studied as indicators of viral pollution in fecally contaminated water. Our objective was to evaluate the distribution of three viral fecal indicators (hAdVs, MS2, and ΦX174), between two different phyllosilicate clays (kaolinite and bentonite) and the aqueous phase. A series of static and dynamic experiments were conducted under two different temperatures (4, 25 °C) for a time period of seven days. HAdV adsorption was examined in DNase I reaction buffer (pH = 7.6, and ionic strength (IS) = 1.4 mM), whereas coliphage adsorption in phosphate buffered saline solution (pH = 7, IS = 2 mM). Moreover, the effect of IS on hAdV adsorption under static conditions was evaluated. The adsorption of hAdV was assessed by real-time PCR and its infectivity was tested by cultivation methods. The coliphages MS2 and ΦX174 were assayed by the double-layer overlay method. The experimental results have shown that coliphage adsorption onto both kaolinite and bentonite was higher for the dynamic than the static experiments; whereas hAdV adsorption was lower under dynamic conditions. The adsorption of hAdV increased with decreasing temperature, contrary to the results obtained for the coliphages. This study examines the combined effect of temperature, agitation, clay type, and IS on hAdV adsorption onto clays. The results provide useful new information on the effective removal of viral fecal indicators (MS2, ΦX174 and hAdV) from dilute aqueous solutions by adsorption onto kaolinite and bentonite. Factors enabling

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enteric viruses to penetrate soils, groundwater and travel long distances within aquifers are important public health issues. Because the observed adsorption behavior of surrogate coliphages MS2 and ΦX174 is substantially different to that of hAdV, neither MS2 nor ΦX174 is recommended as a suitable model for adenovirus.

1. Introduction

Pathogenic enteric viruses are frequently found in natural and wastewaters (Hundesa et al., 2006; Muscillo et al., 2008; Ogorzaly et al., 2009). The presence of pathogenic enteric viruses in the environmental waters poses a significant risk to human health. Their extremely small size (e.g., 23–25 nm for MS2, 28–30 nm for hepatitis A virus, 27–30 for poliovirus, 35–39 nm for norovirus, 65–85 nm for adenovirus) enables enteric viruses to penetrate soils and contaminate groundwater through wastewater discharges, sanitary landfills, septic tanks, and agricultural practices (Chrysikopoulos et al., 2010; Masciopinto et al., 2008; Sim and Chrysikopoulos, 2000; Syngouna and Chrysikopoulos, 2011). Frequently encountered pathogens in environmental systems include adenoviruses, enteroviruses, hepatitis A virus, noroviruses, and rotavirus (Fong et al., 2010; Pang et al., 2014). Among the waterborne viruses, human adenoviruses (hAdVs) are described as emerging pathogens (Jiang, 2006), and they are considered to be highly resistant in water (Ogorzaly et al., 2010). Nevertheless, there is still significant lack of data on the occurrence, persistence and stability of these viruses in groundwater (Mena and Gerba, 2009). To predict the presence of pathogenic viruses in natural waters and wastewater, microorganisms known as indicator organisms (e.g., hAdVs and coliphages), which are commonly associated with fecal contamination, are monitored. HAdVs have been suggested as preferred indicators of viral contamination (Mellou et al., 2014; Poma et al., 2012). Coliphages MS2 and ΦX174 have also been studied as indicators of viral pollution in fecally contaminated water (Grabow, 2001; Havelaar et al., 1986; Chrysikopoulos and Aravantinou, 2012). Compared to other pathogenic viruses, coliphages behave more conservatively (lower sorption); furthermore, they are capable to survive under significant periods of time in groundwater. For this reason, the structural resemblance to many human enteric viruses, coliphages are considered as good model viruses and have been extensively used in numerous studies focused on virus fate and transport in the subsurface (Anders and Chrysikopoulos, 2006; Chrysikopoulos and Aravantinou, 2014; Syngouna and Chrysikopoulos, 2011).

Viruses in natural waters and wastewaters are frequently found attached onto sand, clays, suspended colloids, etc. (Meschke and Sobsey, 1998). Naturally abundant clay minerals are a class of layered alumosilicates, which comprise of various layers of silica and alumina sheets (McBride, 1974), with good biocompatibility, strong adsorption, ion exchange ability and expansibility (Zhang et al., 2010; Zhou and Keeling, 2013; Zhou et al., 2012; Zhou, 2011; Vasiliadou et al., 2011; Vasiliadou and Chrysikopoulos, 2011). Kaolinite, montmorillonite and illite represent some of the main groups of clay minerals found within soils (Brennan et al., 2014). Montmorillonite is 2:1 (3 layer) clays, while kaolinite is 1:1 clay (2 layer) with relatively smaller expansion and adsorption capacity. Montmorillonite, commercially known as bentonite, is most commonly used as an additive to existing natural clay liner materials to decrease permeability (Kau et al., 1998). Bentonite typically displays very low permeability and high cation exchange capacity, which are known to aid in contaminant retardation (Hussin et al., 2011; Ralla et al., 2010).

Virus transport in porous media has found to be influenced by clay colloid presence (Katzourakis and Chrysikopoulos, 2014; Syngouna and Chrysikopoulos, 2013). Moreover, clays have been reported to affect the growth and metabolic activity of viruses. Numerous studies have investigated the interaction between viruses and clays and the effect of clay minerals on virus survival and infectivity (Jin and Flury, 2002; Kimura et al., 2008). Most literature has focused on the effect of clay type (Christian et al., 2006; Lipson and Stotzky, 1985; Templeton et al., 2008), pH (Zhuang and Jin, 2008; Walshe et al., 2010), ionic strength (IS) (Tong et al., 2012), buffer composition (Gutierrez et al., 2010; Zhuang and Jin, 2008), cation exchange capacity (Lipson and Stotzky, 1983; Vettori et al., 1999), virus surface morphology (Block et al., 2014), temperature, and agitation (Syngouna and Chrysikopoulos, 2010). Although Syngouna and Chrysikopoulos (2010) conducted batch experiments to investigate bacteriophage (MS2 and ΦX174) sorption onto various clay minerals at two different temperatures under static and dynamic conditions, to our knowledge, there is no published study that investigated temperature, agitation, IS, and clay type synergistic effects on the interaction of hAdV with clay minerals (kaolinite, and bentonite). Thus, the main objective of this study was to evaluate and compare the adsorption of three viral fecal indicators (hAdVs, MS2, and ΦX174), onto two different phyllosilicate clays (kaolinite and bentonite) under different experimental conditions.

2. Materials and methods

2.1. Cell lines and hAdV stock preparation

The hAdV serotype 35 strain was cultivated in human lung carcinoma cell line A549 growing in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, U.S.) containing 4.5 g/L D-glucose, L-glutamine and pyruvate with 10% heat inactivated fetal bovine serum (FBS; Gibco). A549 cells support the replication of most human adenovirus serotypes, except of the fastidious serotypes 40 and 41. For the preparation of hAdV stocks, A549 cells were cultured confluently (80–90%) in 175-cm^2 flasks, in a CO_2 incubator (5% CO_2) at 37 °C, and infected with hAdV serotype 35 (kindly donated by Dr Annika Allard, University of Umea, Sweden). HAdVs were released from cells by freezing and thawing the culturing flasks for 3 times. A centrifugation step at 3000 ×*g* for 20 min was applied to eliminate cell debris. The supernatant was ultracentrifuged for 1 h at 34,500 ×*g*, re-suspended in PBS, quantified and stored in 10 mL aliquots at −80 °C until use. The initial concentration of each hAdV stock was quantified by real-time PCR (qPCR), and recorded at 10^6 genome copies/mL.

2.2. HAdV nucleic acid extraction

The analytical approach was designed to contain a step of an enzymatic treatment by DNase I, aiming at reducing the detection of false positives by qPCR. DNase I should degrade any viral DNA that is no longer protected by the viral capsid. A volume of 2.5 μL of DNase I (RNase — free, 2000 units/mL, New England BioLabs, Inc.) was added to 137.5 μL of each sample and then all aliquots were incubated at 37 °C for 2 h. Following this incubation period, all samples were immediately placed in a freezer at −80 °C for storage prior to nucleic acid extraction and molecular assay. The samples were thawed immediately prior to the nucleic acid extraction step in which 140 μL of each sample was added (separately) to 560 μL of the Buffer AVL and 5.6 μL of the carrier RNA of a QIAamp viral RNA mini kit (Qiagen). The samples are first lysed under the highly denaturing conditions provided by Buffer AVL to inactivate RNases and to ensure isolation of intact viral RNA. Carrier RNA, added to Buffer AVL, improves the binding of viral RNA to the QIAamp membrane especially in the case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity. The DNase I enzyme presumably denatures in this buffer. The viral DNA