Interaction of human adenoviruses and coliphages with kaolinite and bentonite

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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 enteric viruses to penetrate soils, groundwater and travel long distances within aquifers are important public health issues. Because the observed adsorption behavior of surrogate coliphagesMS2 and ΦX174 is substantially different to that of hAdV, neither MS2 nor ΦX174 is recommended as a suitable model for adenovirus.

Materials and Methods

HAdV stock preparation

- Human Adenovirus serotype 35 as model virus
- Cultivation in human lung carcinoma cell line A549
- The initial concentration of adenoviral stock: 10^8-10^9 genome copies/ml
- Quantification by Real-Time Polymerase Chain Reaction (qPCR)

Real – Time PCR (qPCR) hAdV detection

- Hexon gene was used as the target area
- All samples were tested in duplicate (two neat and two diluted)
- Use of TaqMan Universal PCR Master Mix (Applied Biosystems) and a carry-over contamination prevention system, uracil N-glycosylase (Hernroth et al., 2002)

Coliphages

MS2: an F-specific single-stranded RNA phage with effective particle diameter ranging from 24 to 26 nm
ΦX174: a somatic single-stranded DNA phage with effective particle diameter ranging from 25 to 27 nm
Both coliphages (MS2, ΦX174)were assayed by the double-layer overlay method (Adams, 1959)

Clay minerals

- kaolinite (03584 Kaolinite, Fluka, chemical composition: Al_2O_3 ~37.6%, SiO_2 ~47.3%, Fe_2O_3 ~0.5%, TiO_2 ~0.4%, K_2O ~1.8%, Na_2O ~0.1%, loss on calcination ~12%)

- bentonite (18609 Bentonite, Riedel de Haen, > 90% montmorillonite, chemical composition: SiO_2 59.2%, Al_2O_3 18.5%, Fe_2O_3 5.6%, CaO 2.0%, MgO 4.0%, Na_2O 0.2%, K_2O 0.9%, weight loss on ignition 8.7%).

Batch experiments

- Two different controlled temperatures (4 and 25 °C)
- HAdV: Dnase I reaction buffer (pH 7.6 and ionic strength IS= 1.4, 14, 140 mM)
- Coliphages: PBS solution (pH 7 and ionic strength IS=2mM)
- Static and dynamic batch conditions
- Control tubes (virus in the absence of clay)
- Reactor tubes (virus in the presence of clay: 10 mg/mL)
- Dynamic batch experiments: reactor and control tubes attached to a rotator
- Samples were collected every 24 h for a period of 7 days and centrifuged at 2000g (6000 rpm) for 10 min
- The adsorption ofHAdV, MS2 and ΦX174 onto clays was determinated as:

$$\mathrm{Log}_{10}\frac{N_s(t)}{N_c(0)} = \mathrm{Log}_{10}\frac{N_c(t)-N_r(t)}{N_c(0)} \qquad (1)$$

- $N_c(t)$ is the virus concentration at time t in the control tubes (copies/mL or pfu/mL),
- $N_c(0)$ is the initial virus concentration at t=0 in the control tubes (copies/mL or pfu/mL)
- $N_r(t)$ is the virus concentration at time t in the reactor tubes (copies/mL or pfu/mL),
- $N_s(t)$ is the concentration of viruses sorbed onto clays at time t (copies/mL or pfu/mL).

Note that equation (1) provides the combined concentration of viable and inactivated (non-functional) sorbed viruses.

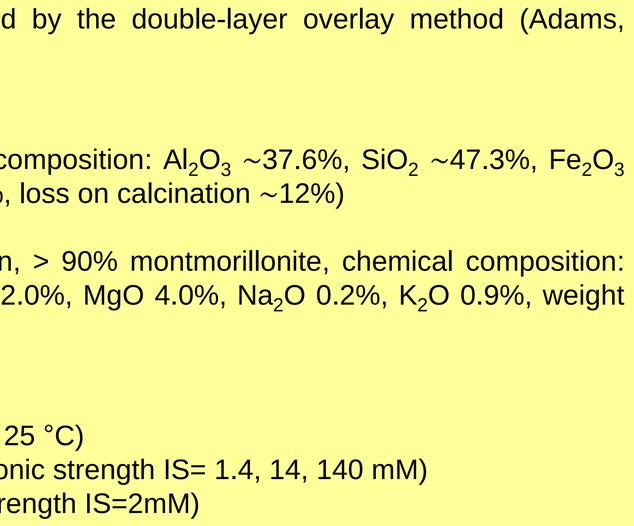

Figure 1. Schematic illustration of the experimental procedure (Bellou et al.,2015).

Results

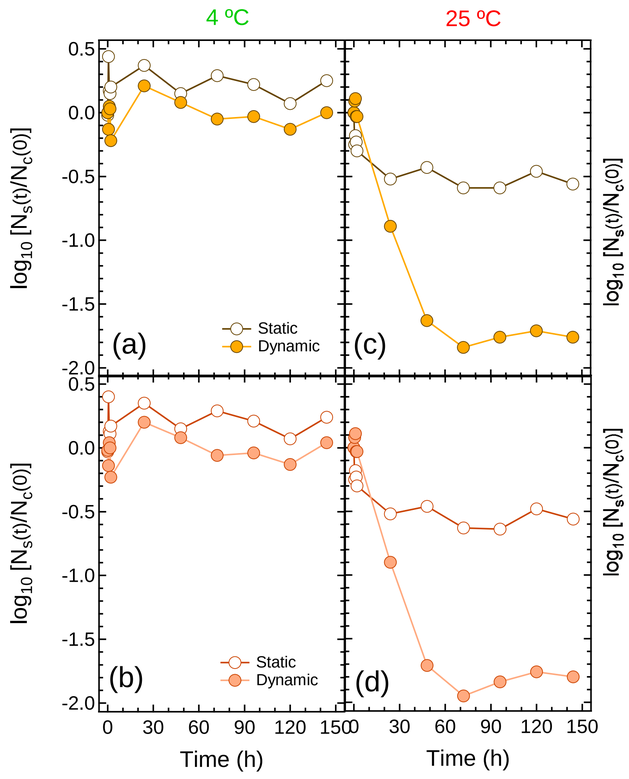

Figure 2. Adsorption of hAdV, expressed as $\log_{10}$ $N_s(t)/N_c(0)$, under static (open circles) and dynamic (solid circles) conditions onto: (a) bentonite at 4 °C, (b) kaolinite at 4 °C, (c) bentonite at 25 °C, and (d) kaolinite at 25 °C.

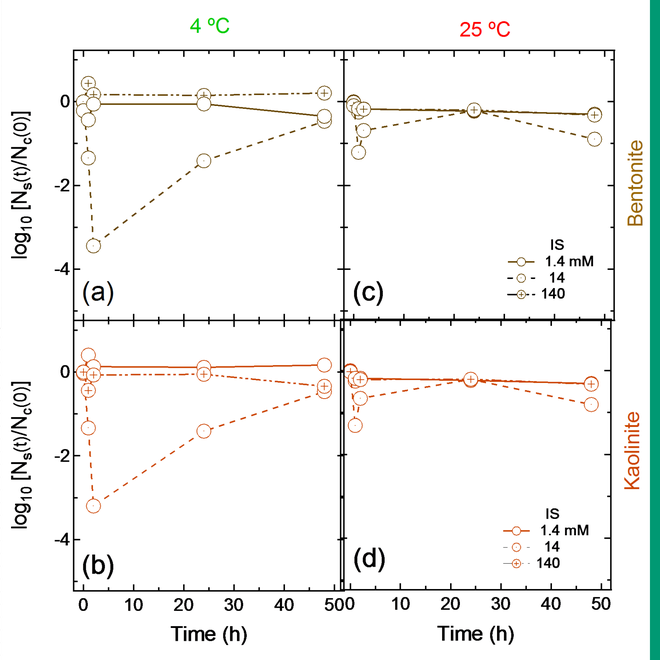

Figure 3. Adsorption of hAdV, expressed as $\log_{10}$ $N_s(t)/N_c(0)$, under static experimental conditions and different ionic strengths (IS=1.4, 14, and 140 mM) onto: (a) bentonite at 4 °C, (b) kaolinite at 4 °C, (c) bentonite at 25 °C, and (d) kaolinite at 25 °C.

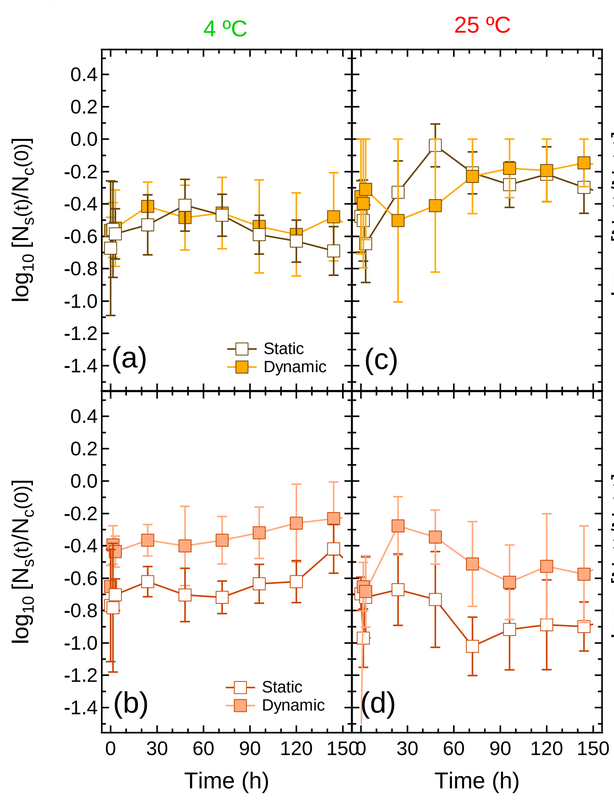

Figure 4. Adsorption of MS2, expressed as $\log_{10}$ $N_s(t)/N_c(0)$, under static (open squares) and dynamic (solid squares) conditions onto: (a) bentonite at 4 °C, (b) kaolinite at 4 °C, (c) bentonite at 25 °C, and (d) kaolinite at 25 °C.

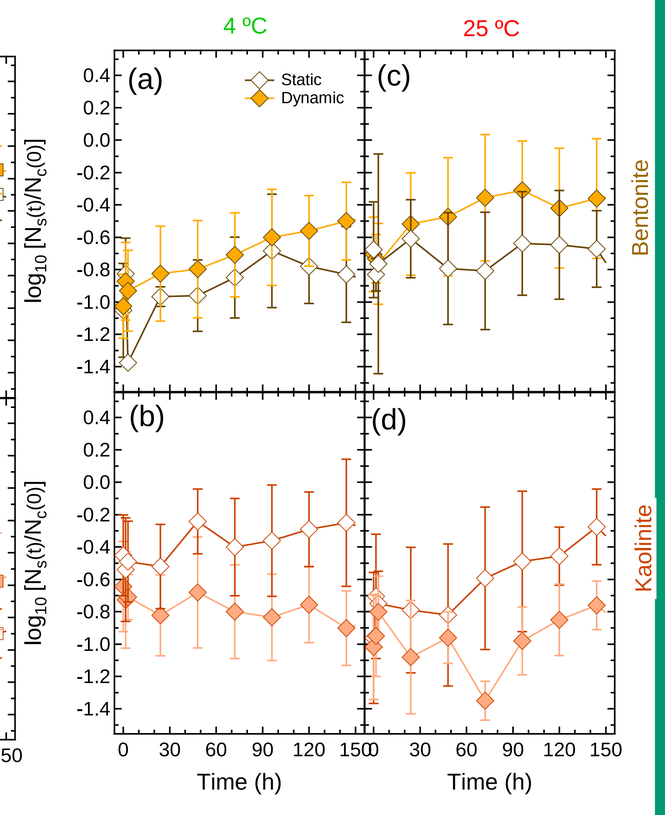

Figure 5. Adsorption of ΦX174, expressed as $\log_{10}$ $N_s(t)/N_c(0)$, under static (open diamonds) and dynamic (solid diamonds) conditions onto: (a) bentonite at 4 °C, (b) kaolinite at 4 °C, (c) bentonite at 25 °C, and (d) kaolinite at 25 °C.

Conclusions

The adsorption of hAdV was found to increase with decreasing temperature, under both static and dynamic batch conditions. For the most of the cases examined, the adsorption of coliphages (MS2 and ΦX174) was shown to increase with increasing temperature, and was higher under dynamic than static conditions. Generally, dynamic conditions improve the contact between viruses and clays, and possibly increase virus adsorption, because the number of accessible sites for attachment is higher. However, hAdV adsorption was lower under dynamic than static conditions, suggesting that agitation either prevents adsorption of hAdV particles or promotes desorption of previously adsorbed hAdV particles. Moreover, for most of the examined cases, hAdV adsorption was found to be higher at IS=140 mM, except for the case of kaolinite at 4 °C, where adsorption was higher at IS=1.4. Note that, hAdV adsorption at three different IS values had a statistically significant difference. The increased reduction of infectious viral numbers due to their contact with clays could play an important role in the prevention of infectious viral waterborne diseases. Finally, the adsorption behavior of surrogate coliphages MS2 and ΦX174 is not similar to that of hAdV. Consequently, neither MS2 nor ΦX174 are suitable models for adenovirus.

References

1. Adams MH. Bacteriophages Interscience. NewYork 1959;450-454.
2. Hernroth BE, Conden-Hansson AC, Rehnstam-Holm AS, Girones R, Allard AK. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. *Applied and Environmental Microbiology* 2002;68(9):4523-4533.
3. Bellou, M.; Syngouna, V.I; Tselepi, M.A; Kokkinos P. A; Paparrodopoulos S. C, Chrysikopoulos C. V.; A. Vantarakis. Interaction of human adenovirus and coliphages with kaolinite and bentonite. *Science of the Total Environment.* 517, 86-95, 2015. http://dx.doi.org/10.1016/j.scitotenv.2015.02.036

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