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Attenuation and colloidal mobilization of bacteriophages in natural sediments under anoxic as compared to oxic conditions

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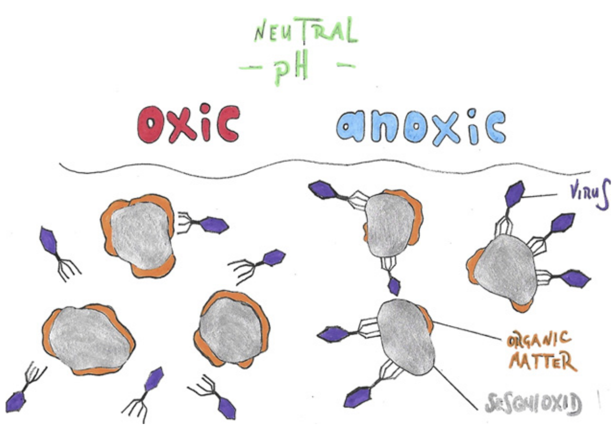
HIGHLIGHTS

- MS2 and PhiX174 did not attach to colloids irrespective of pH and redox potential.
- Phage removal efficiency in anoxic environments depends on organic matter dynamics.
- Organic matter may block Fe/Al-oxides in anoxic sediments.

GRAPHICAL ABSTRACT



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ABSTRACT

Redox conditions are known to affect the fate of viruses in porous media. Several studies report the relevance of colloid-facilitated virus transport in the subsurface, but detailed studies on the effect of anoxic conditions on virus retention in natural sediments are still missing. Therefore, we investigated the fate of viruses in natural flood plain sediments with different sesquioxide contents under anoxic conditions by considering sorption to the solid phase, sorption to mobilized colloids, and inactivation in the aqueous phase. Batch experiments were conducted under oxic and anoxic conditions at pH values between 5.1 and 7.6, using bacteriophages MS2 and PhiX174 as model viruses. In addition to free and colloid-associated bacteriophages, dissolved and colloidal concentrations of Fe, Al and organic C as well as dissolved Ca were determined. Results showed that regardless of redox conditions, bacteriophages did not adsorb to mobilized colloids, even under favourable charge conditions. Under anoxic conditions, attenuation of bacteriophages was dominated by sorption over inactivation, with MS2 showing a higher degree of sorption than PhiX174. Inactivation in water was low under anoxic conditions for both bacteriophages with about one $\log_{10}$ decrease in concentration during 16 h. Increased Fe/Al concentrations and a low organic carbon content of the sediment led to enhanced bacteriophage removal under anoxic conditions. However, even in the presence of sufficient Fe/A-(hydr)oxides on the solid phase, bacteriophage sorption

Abbreviations: PFU, plaque forming units; C_{org}, organic carbon; DOC, dissolve organic carbon.

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was low. We presume that organic matter may limit the potential retention of sesquioxides in anoxic sediments and should thus be considered for the risk assessment of virus breakthrough in the subsurface.

1. Introduction

Human pathogenic viruses which are transmitted via the faecal–oral route, such as enteric viruses, are frequently found in surface water (Hot et al., 2003; Lodder and De Roda Husman, 2005; Albinana-Gimenez et al., 2006; Ngazoa et al., 2008; Westrell et al., 2006). Riverbank filtration, artificial groundwater recharge and slow sand filtration are common methods for the natural (pre-)treatment of drinking water. Riverbank filtration and slow sand filtration have both been reported to remove viruses or virus surrogates (i.e. model viruses such as bacteriophages MS2 or PhiX174) during subsurface passage (Weiss et al., 2005; Bauer et al., 2011). The elimination of viruses is thereby mainly governed by hydraulic settings and physico-chemical parameters of water and sediment along with the surface characteristics of viruses itself (Jin and Flury, 2001).

Redox conditions are reported to play a major role in the transport and inactivation of bacteriophages (Schijven et al., 2000; Van der Wielen et al., 2008). Both authors observed lower virus removal in anoxic than in oxic zones of a natural sandy aquifer which they attributed to lower inactivation and adsorption rates under anoxic conditions. They attribute the reduced virus adsorption to the dissolution of Fe-(hydr)oxides. Similarly, Frohnert et al. (2014) observed lower virus removal under anoxic conditions in a laboratory column experiment during a 4-week-period.

A number of studies indicate the relevance of colloid-associated virus transport along preferential flow paths in sediments (Seta and Karathanasis, 1997; Jin et al., 2000). Colloids are small (1 nm to 10 μm; Brady and Weil, 2002; Stumm and Sigg, 1979) and have a large surface to volume ratio (Kretzschmar et al., 1999). Therefore, colloids tend to be stable in suspensions and are hardly filtered by porous media (Seta and Karathanasis, 1997). These properties make colloids a possible carrier for sorbing viruses (McGechan and Lewis, 2002) which would otherwise be retained by the sediment. Jin et al. (2000) showed that colloid-facilitated MS2-phage transport in porous media was clearly correlated with the amount of transported clay colloids and the number of viruses adsorbed to these. Failure to consider this pathway may result in an underestimation of virus travel velocity, distances and concentrations (McCarthy and Zachara, 1989). To predict the fate and transport of viruses in contaminated waters and sediments, it is important to understand the dynamics of colloid release from sediments and the governing mechanisms of colloid–virus-interactions.

Sorption of viruses to colloids and sediments is controlled by their surface properties, with the surface charge being a key parameter. The charge of individual viruses at a given pH is a function of their isoelectric point (IEP), which varies for different virus types (Jin et al., 1997; pH_{iep}: 3.9 for MS2, and pH_{iep} 6.6 for PhiX174; Dowd et al., 1998). Hence, virus charge is controlled by pH and ionic strength of the solution. Similarly, colloid charge is also a function of IEP (determined by colloid composition) and solution chemistry, such as pH, concentrations of dissolved organic carbon and ionic strength (Kretzschmar et al., 1999).

Emelko and Tufenkje (2010) highlight the need to understand the fate of colloidal pathogens such as viruses in the subsurface (i.e. riverbank filtration systems). However, so far, only few studies looked into the fate of viruses under anoxic conditions (for instance Frohnert et al., 2014). There are many publications on virus retention as a function of pH (Schijven and Hassanizadeh, 2000; Jin and Flury, 2001; Walshe et al., 2010), but they all refer to oxic conditions. In addition, the role of colloid-facilitated virus mobilization or virus transport has never been addressed in this context. Moreover, sesquioxides (i.e. Fe- and Al-(hydr)oxides), which often occur in close association with each other, and their role on virus retention under anoxic conditions is still unclear: non-redox-sensitive sorption sites such as Al-(hydr)oxides could improve virus retention under all redox conditions, but if they are released from the sediment due to break-up of bonds through the dissolution of Fe they may also enhance virus transport if viruses sorb to them. Therefore, the aim of our study was to understand

(i) the dynamics of colloid release from natural flood plain sediments with different sesquioxide contents under anoxic conditions as compared to oxic conditions.
(ii) the fate of viruses in these natural system such as sorption to the solid phase, sorption to mobilized colloids, and inactivation in the aqueous phase

by testing the influence of pH changes on the attenuation of two model viruses, PhiX174 and MS2, in laboratory batch experiments. These two bacteriophages were chosen as they differ in their isoelectric points (Dowd et al., 1998), which is reported to lead to different sorption behaviour (Jin and Flury, 2001).

2. Material and methods

2.1. Origin and characterization of sediments and water

Two sediments were obtained from the Danube floodplain. Sediment 2011 was sampled in an isolated river section with little hydrological connection to the Danube River. This section was silted up with medium-grained fine sand and overgrown with reed and riverine vegetation. Sediment 2016 was sampled in a river section with a pronounced hydrological connection to the Danube, characterized by regular erosion and sedimentation processes and a texture of silty sand. Both samples were mixed and homogenized individually prior to their storage at 4 °C under saturated conditions (i.e. the sediment was always covered with a few centimetres of river water). Both sediments differ strongly in their concentration of organic carbon, Fe and Al (Table 1): sediment 2011 has a high organic carbon (C_{org}) and a low Fe/Al content, sediment 2016 has a low C_{org} and a high Fe/Al content. For our experiments we used treated groundwater (i.e. by removal of Fe and Mn through microbial precipitation) abstracted from a quaternary aquifer on the experimental field site of the Federal Environment Agency in Berlin-Marienfelde, Germany. In order to obtain water of low ionic strength, this groundwater was diluted 1:10 with deionised water, yielding a final ionic strength of about 2 mM, comprising Ca^{2+} and SO_4^{2-} concentrations of about 0.40 mM and 0.25 mM, respectively. The water was autoclaved (20 min at 121 °C) prior to its use to remove any dissolved oxygen and is termed "test water" in the following.

2.2. Bacteriophages

Bacteriophages MS2 (DSM 13767) and PhiX174 (DSM 4497) were used as surrogates of RNA and DNA viruses, respectively. Both viruses develop icosahedral virions with sizes of 25–30 nm. MS2 is an RNA bacteriophage of the family *Leviviridae* with a single-stranded RNA genome, the genome of phage PhiX174 (family *Microviridae*) consists of single-stranded DNA. The isoelectric points given in literature are about 3.9 for phage MS2 and about 6.6 for phage PhiX174 (Michen and Graule, 2010).

To achieve stock suspensions with high bacteriophage concentrations, host bacteria were grown up to exponential growth phase and subsequently inoculated with the respective phages, as described in standardized procedures (DIN EN ISO, 10705-1, 2001; DIN EN ISO, 10705-2, 2001). 10% v/v chloroform was added to destroy the bacteria. After incubation and settling, supernatants were centrifuged (3000 ×g