COTRANSPORT OF VIRUSES AND CLAY PARTICLES IN UNSATURATED POROUS MEDIA

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SUMMARY: Suspended clay particles in groundwater can play a significant role as carriers of viruses, because, depending on the physicochemical conditions, clay particles may facilitate or hinder the mobility of viruses. This experimental study examines the effects of clay colloids on the transport of viruses in variably saturated porous media. All cotransport experiments were conducted in both saturated and partially saturated columns packed with glass beads, using bacteriophage MS2 as a model virus, and kaolinite (KGa-1b) and montmorillonite (STx-1b) as model clay colloids. The various experimental collision efficiencies were determined using the classical colloid filtration theory. The experimental data indicated that the mass recovery of viruses and clay colloids decreased as the water saturation decreased. Temporal moments of the various breakthrough concentrations collected, suggested that the presence of clays significantly influenced virus transport and irreversible deposition onto glass beads. The mass recovery of viruses, based on total effluent virus concentrations, was shown to reduce in the presence of suspended clay particles. Furthermore, the transport of both suspended and attached onto suspended clay-particles viruses was retarded, compared to the conservative tracer. Under the unsaturated conditions both clay particles hindered the transport of MS2.

1. INTRODUCTION

Several theoretical and experimental investigations have shown that suspended mobile colloids in water saturated porous media can either facilitate or hinder the mobility of various contaminants (Abdel-Salam and Chrysikopoulos, 1994; Chu et al., 2003; Syngouna and Chrysikopoulos, 2010, 2013). However, colloid facilitated virus transport in unsaturated porous media is substantially different and more complex than that in saturated porous media. In addition to the retention mechanisms governing colloid and virus transport in saturated porous media (e.g., pore straining and attachment onto solid-water interfaces (SWI)), in unsaturated porous media colloids can be retained at air-water interfaces (AWI), in thin water films (film straining), and air-water-solid (AWS) interfaces (Wan and Wilson, 1994; Corapcioglu and Choi, 1996; Wan and Tokunaga, 1997). Furthermore, in unsaturated porous media colloids and viruses can also be retained in air-water meniscus-solid (AWmS) interfaces (Zevi et al., 2005; Gao et al., 2006). Note that the AWmS interface is essentially the area where significant colloid attachment occurs and the water meniscus diminishes to a thin water film.

The objective of this paper is to explore further the specific interactions of simultaneously transported colloids and viruses in unsaturated porous media with the various interfaces (SWI, AWI, and AWS). Also, the synergistic effects of suspended clay colloids and water saturation level on the attenuation and transport of viruses in unsaturated porous media is examined.

2. MATERIALS AND METHODS

2.1. Bacteriophages and assay

The bacteriophage MS2 (F-specific single-stranded RNA phage with effective particle diameter ranging from 24 to 26 nm) was used as a model virus. MS2 is infecting *E. coli*, and was assayed by the double-layer overlay method, as outlined by Syngouna and Chrysikopoulos (2010). For the separation of viruses adsorbed onto clay particles from suspended viruses in the liquid phase, centrifugation at 2000×g for 30 min was used as described in Syngouna and Chrysikopoulos (2013). The suspension of unattached viruses in the supernatant was pipetted out and the suspended viruses were determined. The concentration of attached viruses was determined by subtracting the mass of viruses that remained in suspension from the initial virus concentration in each sample.

2.2. Clays

The clays used in this study were kaolinite (KGa-1b, a well-crystallized kaolin from Washington County, Georgia) and montmorillonite (STx-1b, a Ca-rich montmorillonite, white, from Gonzales County, Texas), purchased from the Clay Minerals Society, Columbia, USA. The <2 μm colloidal fraction of each clay mineral suspension in sterile ddH2O was separated by sedimentation and was purified. The optical density of the clay colloids was analyzed at a wavelength of 280 nm by a UV-vis spectrophotometer, and the corresponding clay concentrations were determined as outlined by Chrysikopoulos and Syngouna (2012). The hydrodynamic diameter of the clay colloids was measured by a zetasizer (Nano ZS90, Malvern Instruments) and was found to be equal to d_p=843±126 nm for KGa-1b, and d_p=1187±381nm for STx-1b.

2.3. Porous media

Glass beads were used as the packing material of the columns in order to eliminate possible experimental difficulties associated with real soil, which may provide numerous uncertainties that can complicate considerably the analysis of the experimental data. Following the procedure previously described by Syngouna and Chrysikopoulos (2013), the glass beads were purified, then were dried in an oven at 105 °C, and finally stored in screw cap sterile beakers until use in the column experiments.

2.4. Column Experiments

All flow through experiments were conducted using a Plexiglas column with length 15.2 cm and internal diameter 2.6 cm. The experimental setup is similar to that described in detail by Mitropoulou et al. (2013). Briefly, the column was uniformly wet-packed with glass beads. Several pore volumes of the de-aired sterile ddH_2O were passed through the column from the bottom to avoid the capture of air bubbles. The packed column was attached to a vacuum chamber (Soil Measurement Systems, Tucson, AZ) with a fraction collector inside, which allowed for various levels of water saturation. The water potential and the uniformity of water in the column were verified with tensiometer readings, which were collected continuously using a CR800 datalogger (Campbell Scientific, Inc., Logan, UT). Liquid samples were collected at regular time intervals from the column effluent in small fractions with an automatic fraction collector. A fresh column was packed for each experiment. The entire packed column and glassware used for the experiments were sterilized in an autoclave at 121°C for 20 min. Constant flow of ddH_2O at flow rate of Q=1.5 mL/min was used. The mean pH of the column influent remained constant at 7.0±0.2 for the duration of each experiment. One set of experiments was performed with viruses and clay particles alone in order to determine their individual transport characteristics. Another set of cotransport experiments was performed to investigate the effect of the presence of clay colloids on virus transport. Chloride, in the form of potassium chloride (KCl), was chosen as the nonreactive tracer.

3. THEORETICAL CONSIDERATIONS

3.1. Moment analysis

The colloid concentration breakthrough data obtained at the end of the packed column (x=L) were analyzed by the first normalized temporal moments M_1 [t] (James and Chrysikopoulos, 2011). In this study, four different $M_{1(i)}/M_{1(t)}$ ratios of the first normalized temporal moment, indicating the degree of velocity enhancement of colloid (i) relative to the conservative tracer (t), were calculated based on the effluent concentrations: $C_{Total-v}$, C_c, C_v, and C_{vc}. Furthermore, the mass recovery, M_r [-], of the tracer or the suspended particles was quantified Mitropoulou et al. (2013).

3.2. Filtration theory

The classical colloid filtration theory (CFT) was used to quantitatively compare the attachment of viruses and clay particles onto SWIs. The CFT is employed for the estimation of the dimensionless collision efficiency, α [-], which represents the ratio of the collisions resulting in attachment to the total number of collisions between particles and collector grains (Yao et al., 1971). The collision efficiencies, $\alpha_{Total-v}$, based on $C_{Total-v}$ and α_v, based on C_v in the effluent for both MS2 and ΦX174 were calculated for the experimental conditions of this study.

In this study, the suspended clay concentration was indicated by C_c. Also, for the cotransport experiments the total virus concentration, $C_{Total-v}$, was assumed to be equal to the effluent suspended virus concentration, C_v, plus the concentration of viruses attached onto suspended clay particles, C_{vc} ($C_{Total-v} = C_v + C_{vc}$). The tracer, virus, and clay initial concentrations were denoted by C_{t0}, C_{v0}, and C_{c0}, respectively; whereas, the concentrations of clay particles attached onto SWI and AWI are denoted by C_{c^*} and $C_c^{\Diamond}$, respectively. Two different apparent collision efficiencies were calculated. The first collision efficiency, $\alpha_{Total-v}$, is based on $C_{Total-v}$ in the effluent and represents the attachment of C_v onto SWI, AWI, C_c* and $C_c^{\Diamond}$. The second collision efficiency, α_v, is based on C_v in the effluent and represents the attachment of both C_v and C_{vc}, onto SWI and AWI, denoted as C_v^* and C_{vc}^*, $C_v^{\Diamond}$ and $C_{vc}^{\Diamond}$ respectively, as well as the attachment of C_v onto C_c, C_c^* and $C_c^{\Diamond}$, denoted as C_{vc}, C_{vc}^* and $C_{vc}^{\Diamond}$, respectively.

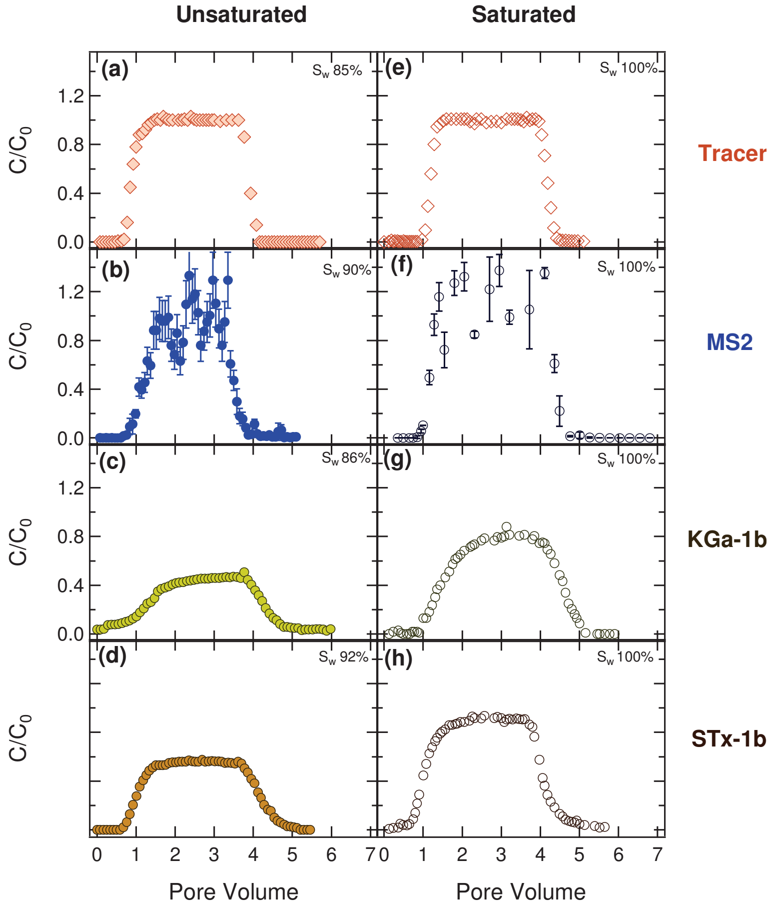

Figure 1. Experimental data of tracer, MS2 virus and clays (KGa-1b, STx-1b) breakthrough in unsaturated (a,b,c,d) and saturated (e,f,g,h) columns packed with glass beads.

4. RESULTS AND DISCUSSION

4.1. Transport experiments

Figure 1 presents the normalized breakthrough data for tracer, MS2 virus and clay particles (KGa-1b, STx-1b) as a function of pore volume for the unsaturated (Figure 1a,b,c,d) and saturated (Figure 1e,f,g,h) column conditions. Although low MS2 M_r values cannot lead to the conclusion that virus particles retained were irreversibly attached or inactivated, the possibility that low M_r values are due to MS2 irreversible attachment and inactivation cannot be excluded. Velocity enhancement (early breakthrough) was observed for viruses and clay colloids for all cases examined. Note that higher velocity enhancement for viruses and clays compared to the tracer was observed under unsaturated than saturated experimental conditions.

Table 1. Experimental conditions and estimated parameters

Exp No	Initial concentration C_{t0}, C_{v0}, C_{c0}	θ_m (-)	S_w (-)	U (cm/min)	M_r (%) for $C_{Total-v}$ or C_c	M_r (%) for C_v	$M_{1(i)}/M_{1(t)}$ for $C_{Total-v}$ or C_c	$M_{1(i)}/M_{1(t)}$ for C_v	$\alpha_{Total-v}$ (-)	α_v
Transport experiments										
Tracer Cl−										
1	0.01moL/L	-	1	0.74	100		1			
2	0.01moL/L	0.38	0.93	0.66	111		1			
MS2										
3	4842 PFU/mL	-	1	0.74	100		1.0		0.00018	
4	13150 PFU/mL	0.38	0.9	0.58	76.6		1.26		0.078	
KGa-1b										
5	67.6 mg/L	-	1	0.74	79.5		1.1		0.114	
6	69.28 mg/L	0.35	0.86	0.7	49.29		1.23		0.529	
STx-1b										
7	102.3 mg/L	-	1	0.74	93.8		1.0		0.018	
8	88.06 mg/L	0.38	0.92	0.69	59.14		1.26		0.252	
Cotransport experiments										
MS2-KGa-1b										
9	9767 PFU/mL	-	1	0.74	53.75	39.98	0.78	0.85	0.109	0.161
	76.98 mg/L				31.41		0.85		0.577	
10	21383 PFU/mL	0.37	0.83	0.72	52.22	25.79	1.08	1.20	0.172	0.360
	69 mg/L				28.64		1.01		0.930	0.172
MS2-STx-1b										
11	9500 PFU/mL	-	1	0.74	67.65	38.45	0.89	0.92	0.069	0.168
	92.56 mg/L				42.88		0.85		0.238	
12	11067PFU/mL	0.35	0.81	0.7	69.98	43.42	1.09	1.00	0.091	0.213
	89 mg/L				22		1.12		0.612	

4.2. Cotransport experiments

Figure 2 presents the normalized breakthrough data for MS2 and clay (KGa-1b, STx-1b) cotransport under both saturated and unsaturated column conditions. The corresponding M_r values, based on $C_{Total-v}$ of MS2 in the effluent, were considerably reduced in the presence of clay particles compared to those obtained in the absence of them (see Table 1). This suggested that some clay-bound viruses were retained in the column due to clay attachment onto the glass beads. Moreover, higher M_r values based on $C_{Total-v}$, C_v, C_{vc} and C_c were observed under saturated than unsaturated experimental conditions. Furthermore, the various $M_{1(i)}/M_{1(t)}$ ratios based on $C_{Total-v}$ of MS2 indicated that $C_{Total-v}$ of MS2 was retarded compared to the tracer only under the saturated conditions. The same trend was observed under saturated conditions for $M_{1(i)}/M_{1(t)}$ ratios based on C_v of MS2. Also, KGa- 1b and STx-1b were retarded compared to the tracer under saturated conditions. Note that under saturated conditions, KGa-1b hindered the transport of MS2 while STx-1b facilitated the transport of MS2. Moreover, under unsaturated conditions both clay particles hinder the transport of MS2.

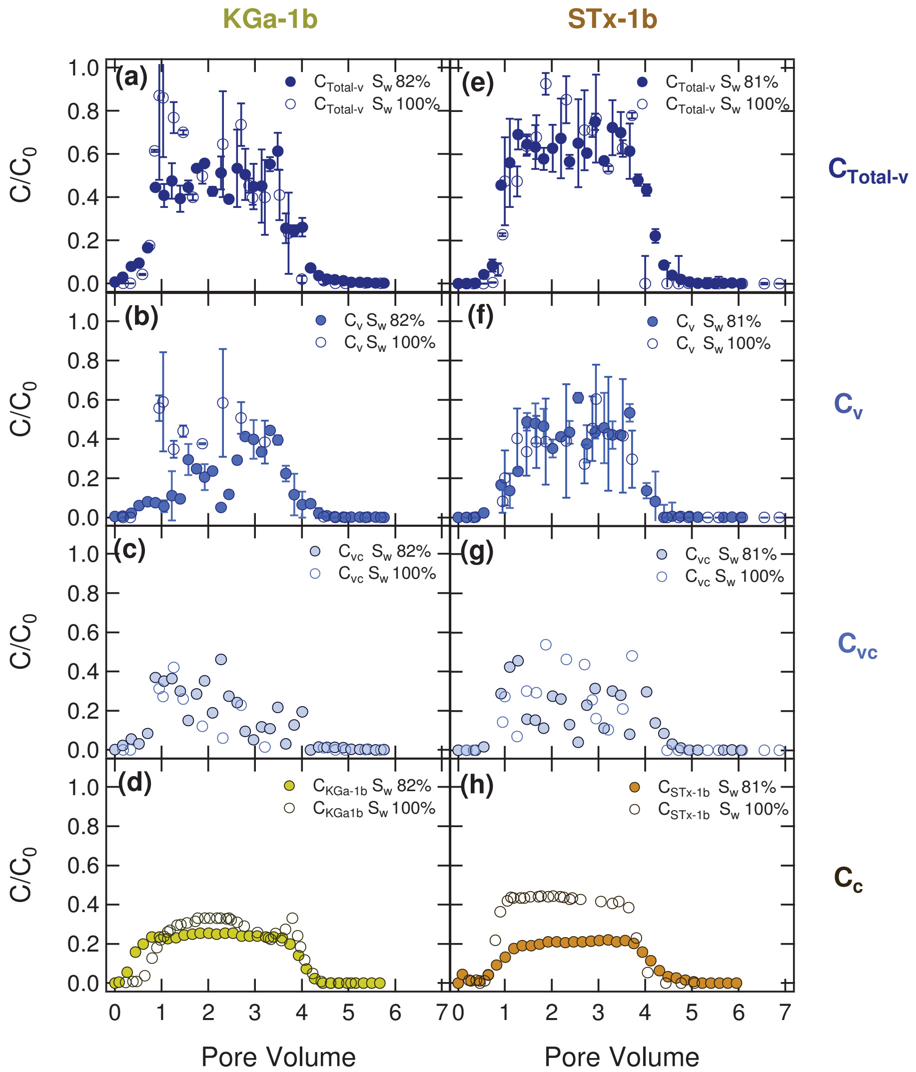

Figure 2. Experimental data for the cotransport of (a,b,c,d) MS2-KGa-1b and (e,f,g,h) MS2-STx-1b in both saturated (open symbols) and unsaturated (filled symbols) columns packed with glass beads.

The various M_r values for MS2, KGa-1b and STx-1b are illustrated graphically in Figure 3. Note that with the only exception of MS2-KGa-1b cotransport, the various Mr values listed in Table 1 indicated that by increasing the saturation level, the difference between $C_{Total-v}$ and C_v for MS2 also increased, suggesting that more viruses were attaching onto suspended clay colloids. Also, the presence of MS2 affected C_c transport because the M_r of both KGa-1b and STx-1b was substantially reduced in the presence of MS2 viruses compared to the case of C_c transport in the absence of them under both saturated and unsaturated conditions (see Table 1). The M_r values for both KGa-1b and STx-1b based on Cc, in most cases examined, were lower under unsaturated than saturated conditions.

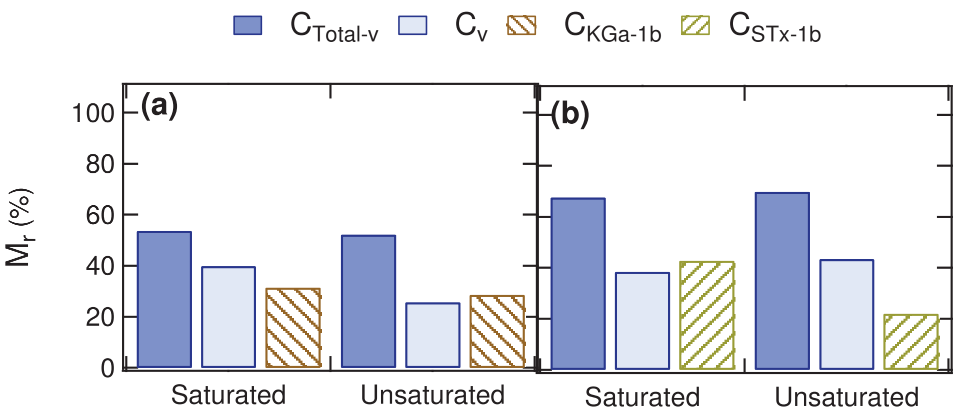

Figure 3. Calculated M_r values based on $C_{Total\text{-}v}$ (solid columns), C_v (filled columns), and C_c (cross-shaded columns) for cotransport of: (a) MS2 with KGa-1b, and (b) MS2 with STx-1b under saturated and unsaturated experimental conditions.

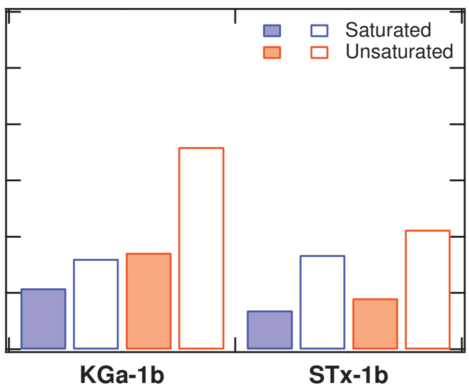

Figure 4. Experimental collision efficiencies $\alpha_{Total-v}$, based on $C_{Total-v}$ (filled columns) and α_v, based on C_v (open columns) in the effluent in both saturated and unsaturated conditions for MS2-clays (KGa-1b, STx-1b) cotransport experiments.

4.3. Collision efficiencies

The collision efficiency values, $\alpha_{Total-v}$, based on $C_{Total-v}$, and α_v values based on C_v, as are presented in Figure 4. In the presence of clay colloids (cotransport experiments), the $\alpha_{Total-v}$ values were higher for MS2 in all cases examined, which indicated that more attachment sites were available on the solid matrix (SWI, AWI, C_c^* and $C_c^\lozenge$) than in the absence of them. In the presence of both KGa-1b and STx-1b, the $\alpha_{Total-v}$ values increased with decreasing saturation level. Furthermore, the relative high α_v values ($\alpha_v > \alpha_{Total-v}$) (see Table 1) indicated that the presence of clay colloids increased the attachment of MS2 viruses onto SWI, AWI and clay colloids. In all cases examined, the αv values increased with decreasing saturation level.

5. SUMMARY AND CONCLUSIONS

In this study, the individual effects of two major parameters (presence/absence of clay colloids, and saturation level) that influence virus and colloid cotransport were examined. The results from this study suggest that the mass recovery of MS2 and clay colloids decrease with decreasing saturation level; whereas, for the cotransport experiments no clear trend was observed. Note that under unsaturated conditions both clay particles hinder the transport of MS2.

ACKNOWLEDGMENTS

This research has been co-financed by the European Union (European Social Fund-ESF) and Greek National Funds through the Operational program "Education and Lifelong Learning" under the action Aristeia I (Code No. 1185). This work is a collaboration between members of the BioMet Network.

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