VIRUS SORPTION AND TRANSPORT IN SATURATED SEDIMENTS AS INFLUENCED BY SALINITY AND SOLUBLE ORGANIC MATTER

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ABSTRACT

In this research, the sorption and transport of MS-2 in saturated sediments under the impacts of salinity and soluble organic matter (SOM) are studied through one-dimensional column experiments. The experiments are conducted with the prepared fine sand (d<sub>50</sub>=0.13mm) from industry and the mixture of the sand and field soil (d<sub>50</sub>=0.16mm) obtained from Bayou Segnette State Park, Louisiana. The controlled conditions in all experiments include constant flow rates (0.2 mm/s), neutral condition (pH=7.0±0.5), and constant temperature (25±0.2°C). Bromide is adopted as the conservative tracer to evaluate the retardation factors of MS-2 in the column. In the sorption and transport experiment with MS-2 mixing with nanopure water, the MS-2 showed slight adsorption effect onto sediments. When SOM was added, no attenuating effect on MS-2 adsorption was observed because of MS-2’s already weak adsorption in nanopure water. The MS-2 with salinity experiment showed dramatically enhanced adsorption effect in the sand column. It can be explained that the salinity compressed double layers, thereby increasing attachment rates. However, no effect was observed in the sand/soil mixture column, counteraction by organic matter from the soil is the main reason.

1. INTRODUCTION

The decline in coastal water quality caused by a rapidly increasing population, caused in part by the disposal of poorly treated domestic wastewater, is a growing concern across the nation. Usually, communities and dwellings in this region have to rely on onsite wastewater treatment because centralized treatment is not feasible due to limited hydraulic gradients and the scattered nature of dwellings. However, the septic system, which is the most widely used onsite wastewater treatment method, usually doesn’t function well due to high water tables, poor soil conditions, or influent flows exceeding the adsorptive capacity of the soil (USEPA, 2002). The consequential threats are generally linked to public health concerns and economic loss. It is reported that about 71% of all waterborne illness outbreaks in the United States since 1990 resulted from pathogen contaminated groundwater (Kramer et al, 1996).

The Marshland Upwelling System (MUS) was developed to replace conventional septic systems in coastal areas for domestic wastewater treatment (Addo et al, 2006; Richardson and Rusch, 2005; Watson and Rusch, 2002). The principle of MUS is to
inject wastewater from coastal dwellings into the subsurface sediment, where the wastewater plume is pushed upward due to the buoyancy force derived by the density difference between wastewater and the native saline groundwater. Pollutants of the wastewater thus can be removed by physical/chemical operations and biological processes during transport. Researchers (Addo et al., 2006; Evans, 2005; Turriciano, 2005; Watson and Rusch, 2002) have demonstrated the ability of the MUS to treat bacteria and nutrients. Corbin (Corbin, 2004) found that the bacteriophage, MS-2, was removed effectively in a MUS system located in Bayou Segnette, Louisiana. Although bacteriophage removal mechanisms of MUS can be generally discussed as adsorption, inactivation, and transport in porous media, the mechanisms are not understood completely.

In the past two decades, numerous batch, column, and field scale studies have been performed to investigate adsorption, inactivation, and transport of various microorganisms in different porous media (Bales et al., 1991; Gannon, 1991; Grant et al., 1993; Penrod et al., 1996; Chu et al., 2003; Guan et al., 2003). Research on virus transport has been of particular interest because of its long transport distance and potential threat to public health. It has been shown that virus type, soil type, pH, ionic strength and multivalent cations, and organic matter can affect the adsorption of the virus, whereas temperature and unsaturated conditions are crucial for virus inactivation (Schijven and Hassanizadeh, 2000; Harvey and Ryan, 2004). However, most of the reported studies have been performed under low soluble organic matter and ionic strength conditions that mimic the characteristics of potable groundwater. Little research has been conducted to investigate the fate and transport of viruses in wastewater moving through high salinity groundwater, which is typical for coastal areas.

The purpose of this work was to investigate the impact of high soluble organic matter (SOM) and high salinity, which represent the wastewater moving in the subsurface sediments of the MUS, on the sorption and transport of viruses. MS-2 was used as a pathogen surrogate owing to its similar morphology and survival characteristics to some important groups of human viruses, such as norovirus (Dawson et al, 2005; Sundram et al, 2002). Results obtained from lab studies would be helped to understand the mechanism of viral sorption and transport of the MUS.

2. MATERIALS AND METHODS

**Column Studies.** Nine continuous-flow 1-D column studies were conducted using bacteriophage MS-2 at pH 7.0±0.5. The temperature was held constant at 25±0.2 °C in a low temperature incubator (Thermolyne, Type 4200). Experiments were performed in 45.3cm (length) × 3 cm (diameter) acrylic columns using prepared sand and a sand/soil mixture representative of sediments in coastal areas. The sand was TECO-sil®Fused Silica, while the soil was from the Bayou Segnette State Park, Louisiana. X-Ray Diffraction analyses showed that quartz, illite, smectite, kaolinite and chlorite are the main minerals in the soil, while quartz is the only mineral in the sand (Figure 1). The organic matter content of the soil was 8.75%. The particle
distribution analysis showed that the sand sample contained 24% coarse sand (0.5-2 mm), 11% medium sand (0.25-0.5 mm), 55% fine sand (0.05-0.25 mm), and 10% silica flint (<0.05 mm). The soil was mixed with the sand (sand/soil mixture) to obtain the similar particle distribution and the mixture has 30% coarse sand, 5% medium sand, 56.5% fine sand, 4.5% silt (0.002-0.005 mm) and 4% clay (<0.002 mm). The mean diameters (d$_{50}$) of the sand and the sand/soil mixture are 0.13 mm and 0.16 mm, respectively. The porosities for both are 37%. The sand was washed with 10% HNO$_3$, 0.5M NaOH and nanopure water, then oven-dried at 90ºC for 20-24 hours prior to packing. To avoid MS-2 adsorption, Teflon tubing was used everywhere in the system except for a length of PharMed® tubing (MasterFlex® 06485-13) in the pump. Before the experiment, each column was placed under vacuum for 2 hours and flushed with about 10 pore volumes of the solutions used in the experiment to establish fully saturated and steady-state flow conditions. MS-2 in nanopure water, SOM water (COD=176, 280 mg/L) and saline water (salinity=10, 20 ppt) were tested both in the sand and the sand/soil mixture columns. For the nanopure water and SOM water experiments, the MS-2 solution was added to each column for three pore volumes using a peristaltic pump. Afterwards, a solution without MS-2 was pumped into the column for 7 pore volumes to flush any remaining MS-2 from the column. For the salinity experiments, the MS-2 solution was added to each column continuously until the end of the experiment. The velocity was controlled at 0.2 mm/s (1.57 pore volumes/hr) for all experiments. Samples were collected from the column effluent using glass vials and stored in an ice chest. An extra salinity level (5 ppt) was performed for the sand column because no MS-2 was flushed out at higher salinity levels. In this experiment, samples were collected both at the top of the column and 15 cm above the influent. MS-2 analyses were performed within 2 hours.

**Figure 1.** The X-ray diffraction curve of sediments. (a) quartz in sand sample; (b) quartz and clay in soil sample; (c) illite, smectite, kaolinite and chlorite in clay sample.

**Materials and Chemicals.** Bromide (potassium bromide at 6-10 mg/L) was used as a conservative chemical tracer to evaluate flow dispersion in the column and analyzed using Ion Chromatography. The soluble organic matter (SOM) was prepared with 0.27g Dextrose, 1.18mL Algal Culture Formula Part A (Kent®), 0.018g sodium phosphate, 0.072g ammonium chloride per liter nanopure water to obtained a COD of
700 mg/L. 0.1M NaOH and 0.5 M HCL were added to adjust the pH of the solution. The soluble organic matter was analyzed as COD by spectrophotometry (HACH DR/4000U). The salt water solutions were prepared by dissolving sea salts (Instant Ocean®) into nanopure water. Salinity was measured using a refractometer.

**Bacteriophage and Assays.** Bacteriophage MS-2 (ATCC 15597-B1) is an icosahedral phage with a diameter of 26.0-26.6 nm and pH_{eq} of 3.9 (Bales and Li, 1993). It was grown and purified in *Escherichia coli* (ATCC 700891). The MS-2 concentration in solution used for the column tests was between 60-600 PFU/mL. Samples were assayed for MS-2 within 2 hours after collection using the plaque-forming-unit method (Adams, 1959). All samples assay were performed duplicate and the mean was reported.

**Data Analysis.** This research focused on the change of the MS-2 retardation factor as influenced by SOM and salinity. Under the assumption of a linear isotherm, the retardation factor is the ratio of the time of the breakthrough curve of a solute to that of a conservative tracer. The mean retention time (MRT) was considered as the following (Levenspiel, 1999):

\[
T = \frac{\int_0^t \tau \, d\bar{C}}{\int_0^1 d\bar{C}}
\]

(1)

where \(\bar{C}\) is the normalized concentration (dimensionless) \((\bar{C} = C / C_0)\); and \(\tau\) is pore volume normalized time (dimensionless). The solute in this study is MS-2, and the conservative tracer is bromide. The retardation factor RF was calculated as (Richardson et al, 2004):

\[
RF = \frac{T}{T_{Br^-}}
\]

(2)

where \(T_{Br^-}\) is the MRT of bromide.

**3. RESULTS**

The breakthrough curves of MS-2 and bromide under different experimental conditions are illustrated in Figures 2 to 5, while calculated MRT and RF values are listed in Table 1.

Experiments were first conducted with the MS-2 and bromide suspended in nanopure water. The breakthrough curves of MS-2 (Figure 2(a)) reached \(C/C_0=1\) at approximately 2.0 pore volumes, resulting RFs of 1.33 and 1.04 for the sand and sand/soil mixture columns, respectively. These results indicated a somewhat greater MS-2 adsorption effect in the sand than that in the soil/sand mixture. When MS-2 injection was stopped and flushing continued, the MS-2 concentration dropped quickly in both columns and all MS-2 were flushed out after about 5-6 pore volumes. However, breakthrough of MS-2 in the sand/soil mixture column occurred earlier and ended later than that in the sand column. Weaker adsorption and greater dispersion are the two main reasons for this phenomenon. The greater dispersions in the...
sand/soil mixture can be observed from the breakthrough curve of bromide (Figure 2(b), 3(b), 4(b)). The bromide appeared earlier and ended later in the sand/soil mixture, which indicates a higher dispersion effect than that in the sand column.

Figure 2. The breakthrough curves of MS-2 with bromide in nanopure water. (a) MS-2 and (b) bromide.

Figure 3. The breakthrough curves of MS-2 and bromide exposed to low SOM (COD=176 mg/L) water. (a) MS-2 and (b) bromide.

Figure 4. The breakthrough curves of MS-2 and bromide exposed to high SOM (COD=680 mg/L) water. (a) MS-2 and (b) bromide.
Figure 5. The breakthrough curves of MS-2 in saline water. (a) MS-2 in the sand/soil mixture column, salinity = 10 ppt and 20 ppt. (b) MS-2 in the sand column, sampling position: 15 cm above the influent, salinity = 5 ppt.

Table 1. Mean retention times (MRT) in pore volume and retardation factors (RF) for column studies.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Sand</th>
<th>Sand/soil mixture</th>
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<tbody>
<tr>
<td></td>
<td>Bromide</td>
<td>MS-2</td>
</tr>
<tr>
<td>(1) MS-2 in nanopure water</td>
<td>1.07</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>RF 1.33</td>
<td>1.04</td>
</tr>
<tr>
<td>(2) MS-2 in SOM water COD = 176 mg/L</td>
<td>1.09</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>RF 1.03</td>
<td>0.88</td>
</tr>
<tr>
<td>(3) MS-2 in SOM water COD = 680 mg/L</td>
<td>1.01</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>RF 1.14</td>
<td>1.04</td>
</tr>
<tr>
<td>(4) MS-2 in saline water Salinity=10 ppt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RF -</td>
<td>1.01</td>
</tr>
<tr>
<td>(5) MS-2 in saline water Salinity=20 ppt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RF -</td>
<td>1.01</td>
</tr>
</tbody>
</table>

The second and third experiments were performed to test the effect of SOM levels (COD = 168 and 680 mg/L) on the adsorption of MS-2 in the sand and sand/soil columns. According to Figures 3(a) and 4(a), the MS-2 showed similar behavior with MS-2 in Figure 2 (a). This indicates the SOM has little effect on the adsorption of MS-2 in the sand and sand/soil columns. The RFs were 1.03 and 1.14 in the sand column, and 0.88 and 1.04 in the sand/soil column under two SOM levels. These results show no dramatic difference with those obtained in nanopure water.

The last experiment was conducted under different salinity conditions (10 and 20 ppt) by adding salt (Instant Ocean®) into the solution of MS-2 and bromide. From the breakthrough curve (Figure 5(a)), it can be seen that the adsorption of MS-2 didn’t change much in the sand/soil column. The RFs are both 1.01 under the conditions of salinity 10 and 20 ppt, which has no dramatic difference with that in nanopure water.
However, in the sand column, no MS-2 was flushed out until 30 pore volumes for these two salinity levels, which indicates the adsorption of MS-2 was enhanced dramatically. An extra salinity level (5 ppt) was tested for the sand column and samples were collected both from the effluent and 15 cm above the influent of the column. Figure 5(b) shows the breakthrough curve of MS-2 at the point of 15 cm above the influent. It can be seen that after 20 pore volumes, the MS-2 concentration only reached to 25% to 30% of initial concentration. No MS-2 was detected from the effluent of the column.

During all of the experiments, the samples were collected at 5 pore volume intervals from the solution used for the column studies to test the inactivation of MS-2. The results showed that the inactivation during these studies is not distinct and can be ignored.

**4. DISCUSSION**

The retention of microorganisms in porous media has been attributed to several mechanisms: straining or filtration at pore constrictions, adsorption onto surface of sediments, sedimentation in the pores, interception, systematic and random motion, growth and die-off (Corapcioglu and Haridas, 1984). Because of its small size, the main retention mechanism for MS-2 is adsorption onto the surface of the sediments. There are three kinds of adsorption: physical, chemical and exchange adsorption (Weber, 1972). Usually, physical adsorption results from Van der Waals forces. Chemical adsorption is formed by the chemical interaction with the adsorbent. Exchange adsorption is caused by electrostatic attraction to charged sediments. Herzig et al. (1970) indicated that Van der Waals forces could overcome the random movement energy of Brownian motion when the particle diameter is greater than 0.1 µm. As for MS-2, because of its small size (26-26.6 nm), physical adsorption can be ignored. Chemical adsorption can also be ignored when the porous media is non-chemically reactive (Weber, 1972). Thus electrostatic attraction becomes the key adsorption mechanism for MS-2.

The surfaces of the sand and soil generally carry negative electrostatic charges. These charges may be permanent (not pH dependent) or may depend on the pH of the surrounding electrolyte solution (Gannon, 1991). In the neutral solution (pH = 7), the pH_iep of MS-2 is 3.9, which causes MS-2 to be negatively charged. As a consequence, the MS-2 adsorption is expected to be low. The results of this study were in agreement with this expectation. Negligible sorption of MS-2 in soils has also been reported by other researchers (Jin et al, 1997; Bales and Li, 1993).

The existence of SOM is generally considered to decrease virus adsorption when moving through sediments. Usually, the SOM is negatively charged, which compete with viruses for the same binding site (Gerba, 1984). Once the adsorption capacity of the sediments is filled, viruses can not be adsorbed by sediments. However, no obvious SOM effect on MS-2 adsorption was observed in this study. The weak adsorption of the MS-2 is the main reason to explain that. The sand and sand/soil
mixture used in this study are both weak adsorbents for MS-2 and the effect of SOM was thus blinded.

The ionic strength is another important factor that can affect the adsorption of MS-2 onto the sediment. According to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, higher ionic strengths compress the double layers, thereby increasing attachment rates (Schijven and Hassanizadeh, 2000). Further, multivalent cations can link viruses and adsorbents of like charge by forming salt bridges between them (Moore et al., 1981) or by charge reversal (Grant et al., 1993). Many studies have observed the ionic strength effect in the batch and column studies, e.g. (Grant et al., 1993; Penrod et al., 1996). In this research, we also observed that the saltwater dramatically enhanced the adsorption of MS-2 in the sand. However, in the sand/soil mixture column, no obvious ionic effect was observed. That because the existence of organic matter in the soil counteract the salinity effect. Generally, the effect of organic matter in the soil was found to be ambiguous. On one hand, a soil with bonded organic matter has fewer sites for virus adsorption based on its negative charge. In addition, some of the soil organic matter, particular lower-molecular-weight fractions, is soluble and easy to pass into solution. This dissolved organic matter will compete for the same binding sites with viruses and break the salt bridges formed between the virus and multivalent cations, thus decreasing viral adsorption further. On the other hand, the organic matter in the soil may provide a hydrophobic adsorption site, thus increasing viral adsorption (Schijven and Hassanizadeh, 2000). However, as a general trend, the effect of organic matter is dominated by electrostatic rather than hydrophobic interactions (Zhuang and Jin, 2003). In the sand/soil mixture column of this study, the effect depended on the combination of soil type, virus type, salinity and nature of organic matter. Although salinity might increase the MS-2 adsorption by compressing the double layer and forming salt bridges, the existence of organic matter in the soil counteracted such enhancing adsorption by providing fewer adsorption sites, competing with MS-2 for binding site, and breaking the salt bridge.

5. CONCLUSIONS

1-D Column experiments were conducted to investigate the impacts of soluble organic matter and salinity on the adsorption of MS-2 onto the sand and the sand/soil mixture. The following conclusions may be made: (1) Adsorption of MS-2 is sediment dependent. In general, the effect of MS-2 adsorption is not distinct because of the same negative charges for both MS-2 and sediments; (2) The existence of soluble organic matter has no obvious effect on MS-2 due to its weak adsorption onto sediments in nanopure water; (3) Salinity (ionic strength) can compress double layer and hence enhance the adsorption effect of MS-2 onto sands. The effect of salinity is not obvious because of the existence of organic matter in the sand/soil mixture.
ACKNOWLEDGEMENTS

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