

Sorption of MS2 Bacteriophage to Layered Double Hydroxides: Effects of Reaction Time, pH, and Competing Anions

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ABSTRACT

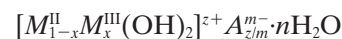
Batch sorption and column breakthrough studies were conducted to investigate the potential of layered double hydroxides (LDHs) to remove bacteriophage MS2 from contaminated waters. All four of the LDHs evaluated in this study had very high retention capacities for MS2. Sorption results showed that MS2 could be completely removed from 5.2×10^2 plaque-forming units (pfu)/mL solution by Mg–Al LDH 2 (i.e., 2:1 Mg to Al ratio LDH), with the highest sorption capacity observed in this study of 1.51×10^{10} pfu/g. Attachment of MS2 to LDHs was a rapid process and reached quasi-equilibrium after a 1-h reaction time. Within the pH range studied (pH 4–9), Mg–Al LDH 2 showed high sorption potential for MS2 at all pH values but sorption decreased slightly with increasing solution pH. Background solution anions influenced virus sorption, with SO_4^{2-} and HPO_4^{2-} decreasing sorption significantly whereas the presence of NO_3^- had little effect on the attachment of MS2 to Mg–Al LDH 2. The addition of another virus (ϕX174) only caused a slight decrease in the retention of MS2 by Mg–Al LDH 2, suggesting that there was insignificant competitive sorption between MS2 and ϕX174 on LDH surfaces. Results from column experiments indicate that there was no MS2 breakthrough from columns packed with Mg–Al LDH 2-coated sand, suggesting complete MS2 retention at the virus concentration tested. The high mass recovery by beef extract solution revealed that the removal of viruses by the LDH was due to sorption of MS2 to LDH surfaces, rather than inactivation.

THE OCCURRENCE OF HUMAN ENTERIC VIRUSES in drinking water constitutes a serious threat to public health and safety. To protect humans from microbial contamination, the USEPA has proposed a set of new regulations to reduce the public health risk resulting from pathogenic contamination (USEPA, 2000). Scientists are working on developing various physical, chemical, and biological means to effectively remove or inactivate viruses from drinking water supplies. Sorption of suspected microbial infectious agents by solid materials is known to be an effective method to quickly remove microbial contaminants from drinking waters (Gerba, 1984; Gupta and Chaudhuri, 1995). The required removal efficiency for viruses from publicly owned drinking water treatment facilities is 99.99% (USEPA, 1989). This strict regulation, and the increasing concern over pathogenic microbial contamination of drinking waters, requires additional research efforts to develop and test natural or synthetic materials as filtration–sorption media to effectively remove viruses. Previous studies have

suggested that some materials such as coal-based media, clay minerals, metal oxides–hydroxides, and activated carbon could be used for virus removal. For example, 70% of bacteriophages (T4 and MS2) were removed from an aqueous solution by bituminous coal (Oza and Chaudhuri, 1975, 1976). Gupta and Chaudhuri (1995) reported that some coal-based media (bituminous coal and lignite treated with aluminum or ferric hydroxide) have very high sorption capacities for polioviruses and that sorption by these materials is very rapid. Clay minerals, kaolinite, and montmorillonite have also been found to have appreciable sorption capacities for reovirus, coliphages (T1 and T7), and bacteriophage MS2 (Schiffenbauer and Stotzky, 1982; Steven and Stotzky 1983, 1984). Atherton and Bell (1983a,b) reported that alkali-treated magnetite had high retention capacities for bacteriophage MS2. The removal of bacteriophages T4 and MS2 was also achieved by sorption to activated carbon (Cookson, 1967; Powell et al., 2000).

Viruses are small (e.g., usually 20–200 nm in size) and consist of nucleic acid encapsulated in a protein capsid. The capsids are composed of protein polypeptides that contain weakly acidic and basic groups (e.g., carboxyl and amino moieties) that are ionizable (Gerba, 1984). Generally, viruses behave as charged colloid particles in aqueous suspension because of ionization of the protein coat. Electrostatic interactions between viruses and solid surfaces are very important in influencing sorption of viruses to sorbents. Under most natural environmental pHs, viruses usually are negatively charged. Therefore, acidification is generally required to assist sorption of viruses on negatively charged materials due to electrostatic repulsion. Positively charged materials are thus considered to be ideal sorbents for removing viruses from aqueous systems.

Layered double hydroxides (LDHs) possess positively charged surfaces; therefore, they may be ideal materials for removing viruses from contaminated waters. Layered double hydroxides are layered solids that are stacked, positively charged octahedral sheets. The general formula of LDHs is:



where M^{II} and M^{III} are bivalent and trivalent cations in octahedral positions and A^{m-} is an anion positioned between the interlayers (Fig. 1). The net positive charge is due to the isomorphous substitution of trivalent metals by divalent metals that is balanced by anions in the interlamellar space. Layered double hydroxides are rare in nature. However, they can be readily synthesized by

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Abbreviations: AGW, artificial ground water; LDH, layered double hydroxide; pfu, plaque-forming units; SEM, scanning electron microscope; TAEC, theoretical anion exchange capacity.

coprecipitation of a solution of bivalent and trivalent metal salts with base (usually NaOH or KOH) under laboratory conditions (You et al., 2001a,b). Layered double hydroxides have relatively large surface areas (0.02–0.12 km²/kg) and high anion exchange capacities (200–500 cmol/kg) (Miyata, 1983; Cavani et al., 1991). Due to their unique properties, there has been increasing interests in investigating LDHs as potential sorbents for removing toxic anionic species, such as CrO₄²⁻, SeO₄²⁻, TeO₄²⁻, dicamba, and 2,4-D, from aqueous systems (Rhee et al., 1997; Goswamee et al., 1998; Kang et al., 1999; Lakraimi et al., 1999; You et al., 2001a, 2002a). The opposite charge properties of LDHs and viruses suggest that LDHs may have the potential to remove viruses from contaminated waters.

In the present study, different LDHs were synthesized by a coprecipitation method. The products were used in batch-sorption tests and column experiments to evaluate their potential for removing water-borne viruses. A bacteriophage, MS2, was selected as the model virus in these experiments because of its structural resemblance to many human enteric viruses that may be found in sewage (Yates et al., 1985) and because it has been used as a surrogate in many studies. Previous studies (Gerba et al., 1981; Herbold-Paschke et al., 1991; Bradford et al., 1993; Farrah and Preston, 1993; Jin et al., 1997; Schijven and Hassanizadeh, 2000) have shown that MS2 generally exhibits poorer sorption behavior to solid surfaces than other viruses such as polioviruses, echovirus Group 7, coxsackievirus B3 and B5, and bacteriophages T2, T4, and φX174. Therefore, using MS2 as a surrogate should provide a conservative estimate of the sorption capacity of LDHs for other enteric viruses and bacteriophages. The main objective of this study was to test the effectiveness of LDHs as sorbents for MS2. Effects of solution pH, reaction kinetics, and presence of competing anions and additional virus species on sorption of MS2 by LDHs were also studied. Results of this research provide important information to guide the potential utilization of LDHs as filtration-sorption materials in point-of-use water treatment devices or on microbial contaminant remediation practices.

MATERIALS AND METHODS

Preparation of Magnesium–Aluminum Layered Double Hydroxides

Magnesium–aluminum (Mg–Al) LDHs were prepared following the method described by You et al. (2001b). Briefly, mixed aqueous solutions of MgCl₂ and AlCl₃ (total metal concentration of 1 M) were coprecipitated by adding dropwise a NaOH solution (2 M) to the mixture at 25 ± 1°C. The molar ratio of Mg to Al ranged from 2.0 to 5.0. Nitrogen (N₂) gas was bubbled throughout the coprecipitation process to minimize CO₃²⁻ in solution. After coprecipitation, the suspensions were stirred for 6 h with pH adjusted to 10 ± 0.3 and were then heated at 65°C for 16 d. After cooling to room temperature, bottles containing the suspension were centrifuged and the precipitates were washed extensively using distilled water. The products were again dried at 65°C, ground, and stored in plastic bottles. Chemical composition of the synthesized Mg–Al

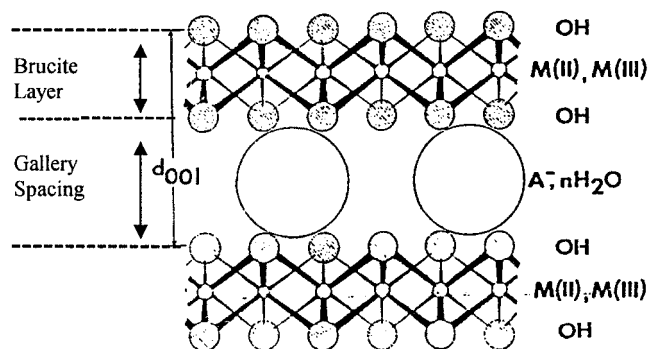


Fig. 1. Schematic representation of a layered double hydroxide structure (after Clearfield et al., 1991).

LDHs was determined using inductively coupled plasma (ICP) spectrometry. Scanning electron microscope (SEM) images of synthesized Mg–Al LDHs were taken using a Hitachi (Tokyo, Japan) S 4700 FE SEM. For the purpose of this study, the products were named Mg–Al LDH 2, Mg–Al LDH 3, Mg–Al LDH 4, Mg–Al LDH 5, with the number 2, 3, 4, or 5 representing the Mg to Al ratio.

Virus and Plaque Assay

Bacteriophage MS2 is an icosahedral single-stranded RNA phage with a diameter of 26.0 to 26.6 nm (VanDuin, 1988) and has an isoelectric point (pI) of 3.9 (Zerda, 1982). The electrostatic potential of MS2 is -17.7 ± 2.3 mV in 0.01 M NaCl solution at neutral pH. The MS2 surface is negatively charged under most natural environmental pHs (Redman et al., 1997). MS2 was grown on bacterial lawns of *Escherichia coli* from the American Type Culture Collection (ATCC 15597) by the agar-overlay method (Adams, 1959, p. 1–4). MS2 enumeration was performed by the plaque assay method described by Adams (1959, p. 1–4) with the aforementioned bacterial host. Briefly, 1 mL of host culture and 1 mL diluted virus sample solution (concentration range between 30 and 300 pfu/mL) were added to trypticase soy agar (TSA) tubes and the mixtures poured onto TSA plates. The plates were solidified for 15 min and incubated for 16 h at 37°C. We only used plates that had 30 to 300 plaques per plate because these are easily and accurately enumerated. All virus assays were performed in duplicate.

Batch Sorption Experiments

A set of batch experiments was conducted to investigate the sorption potential of LDHs for MS2 and to determine the effects of selected environmental factors (pH, reaction time, competing anions, and coexisting φX174 virus) on MS2 sorption. MS2 stock solution was diluted from a concentrated titer with an artificial ground water (AGW; 0.075 mM CaCl₂, 0.082 mM MgCl₂, 0.051 mM KCl, and 1.5 mM NaHCO₃) to desired concentrations. All experiments were conducted at pH 7.5 except for those targeting the effect of pH on virus sorption. First, 5 mL of 180-mg/L LDH stock solution (ultrasonically dispersed) was placed into sterilized 25-mL screw-top Corex glass centrifuge tubes. To avoid the effect of an air–water interface (AWI) (Thompson et al., 1998), approximately 25 mL MS2 solution was carefully added to tubes to form a water convex; caps were gently screwed onto the tubes to avoid air bubbles. Tubes with air bubbles were redone. Once the tubes were properly prepared, they were then shaken in an end-over-end shaker at 20 rpm for 3 h at 4 to 7°C, followed by centrifugation at 9000 × g for 15 min. For sorption kinetics

study, the tubes were shaken for a set of desired contact times. The effect of competing anions (NO_3^- , SO_4^{2-} , and HPO_4^{2-}) on virus sorption was conducted in AGW buffer solution with anion concentrations of 0.025 M. The effect of coexisting bacteriophage ϕX174 on MS2 sorption was performed at two ϕX174 concentrations (10^3 and 10^4 pfu/mL). Virus concentrations in suspensions were determined by plaque assay. All experiments were conducted with three replicates and run at the same time along with blank treatments.

Column Experiments

To test MS2 sorption on LDHs in flow-through situations, column experiments were conducted on both oxide-removed sand and Mg–Al LDH 2-coated sand. The oxide-removed sand was obtained using the method described by Zhuang and Jin (2003). The Mg–Al LDH 2-coated sand was prepared by an autoclave method that is described briefly below. First, 1000 g of oxide-removed sand was placed into a plastic beaker and 2 L of a 5% Mg–Al LDH 2 solution was added. The mixture was autoclaved for 1 h and oven-dried at 80°C with occasional stirring. The Mg–Al LDH 2-coated sands were washed extensively with distilled water to remove unbound Mg–Al LDH 2 and oven-dried before use in the column experiments. Scanning electron microscope images were taken to verify the success of the coating process.

Column transport experiments were conducted using acrylic columns (3.5 cm in diameter and 8 cm in length) under saturated flow conditions at 4 to 7°C (Jin et al., 2000). Each column contained top and bottom plates that were sealed with O-rings. A stainless steel screen was placed on the bottom plate to prevent loss of sand particles. A constant flow rate was maintained using a peristaltic pump. Teflon tubing was used throughout the system except for a portion of Tygon tubing in the peristaltic pump. Previous tests indicated that no virus sorption–inactivation occurred due to the use of acrylic columns and other materials in the system (Chu et al., 2000). Sand was packed in 1-cm increments into the columns, which was pre-filled with deaerated buffer solution (AGW) and stirred to prevent layering and air entrapment. About 100 pore volumes of buffer solution were pumped into the columns to establish a steady state flow and to standardize the pH and ionic strength condition before switching the input to a virus solution. Viruses were introduced into the column by pumping the virus solution (approximately 10^5 pfu/mL MS2 with 50 mg/L KBr as a conservative tracer) as a step function. Outflow samples were collected in 5-mL glass tubes using a fraction collector. The concentrations of MS2 and Br^- in effluent samples were determined by plaque assay and ion chromatograph, respectively. After virus input was ceased, approximately 1200 mL of 3% beef extract solution of pH 9.5 was used to elute the column immediately to distinguish whether the retained viruses was due to sorption of MS2 on LDH surfaces or inactivation because of the presence of the LDH. Beef exact is a high-ionic-strength enzyme digest of beef pro-

teins composed of polypeptones, polypeptides, and amino acids that can detach sorbed viruses from solid surfaces (Gerba et al., 1984). Virus concentrations in the eluted samples with the beef extract solution were determined by the same plaque assay method described earlier and used to calculate the mass recoveries for both columns.

RESULTS AND DISCUSSION

Properties of Synthesized Layered Double Hydroxides

Chemical compositions and selected properties of the synthesized LDHs are presented in Table 1. The Mg to Al ratios of synthesized LDHs conform well to the values prepared in the respective mixed aqueous solutions, suggesting that Mg^{2+} and Al^{3+} quantitatively precipitated during the coprecipitation processes. The empirical formulas of the LDHs calculated from the chemical composition considering Cl^- as the interlayer anion are listed in Table 1. Surface areas of synthesized LDHs ranked as Mg–Al LDH 2 > Mg–Al LDH 3 > Mg–Al LDH 4 > Mg–Al LDH 5. Theoretical anion exchange capacities (TAECs) of the LDHs, which were calculated based on the LDH empirical formula using the method described by Sparks (2002), decreased as the Mg to Al ratio increased in the synthesized LDHs (Table 1).

X-ray diffraction analysis was performed to determine the crystal layer structure of LDHs (You et al., 2001b). A representative X-ray diffraction pattern of Mg–Al LDH 2 is shown in Fig. 2. The X-ray diffraction patterns of synthesized LDHs consisted of sharp and symmetrical peaks, indicating that synthesized LDHs had typical and well-formed layered structures with high degrees of crystallinity. The *d*-spacing values (d_{003}) of the LDHs studied (Table 1) agree well with those reported by others (Miyata, 1975; Clearfield et al., 1991). A representative SEM image of Mg–Al LDH 2 indicates that synthesized Mg–Al LDH 2 is highly crystalline and composed of well-defined hexagonal plate-shape crystals approximately 100 nm in diameter (Fig. 2). Sample was devoid of amorphous material and no crystalline impurities were detected.

MS2 Sorption on Layered Double Hydroxide 2

The ability of Mg–Al LDH 2 to sorb MS2 was tested at different virus concentrations. Artificial ground water with an ionic strength of 0.002 M was chosen as a fluid matrix for suspending the MS2 virus because it represents typical ground water conditions (Saiers and Horn-

Table 1. Chemical compositions and selected properties of synthesized layered double hydroxides (LDHs).

Sample	Mg	Al	$\text{H}_2\text{O}^\dagger$	Empirical formula ‡	<i>d</i> spacing	Surface area §	TAEC $^\parallel$
Mg–Al LDH 2	20.1	11.1	13.2	$[\text{Mg}_{2.0}\text{Al}(\text{OH})_{6.0}]\text{Cl}\cdot 1.8\text{H}_2\text{O}$	7.71	49.1	411.7
Mg–Al LDH 3	22.5	8.73	12.9	$[\text{Mg}_{2.9}\text{Al}(\text{OH})_{7.8}]\text{Cl}\cdot 2.2\text{H}_2\text{O}$	7.94	48.9	323.6
Mg–Al LDH 4	24.2	6.92	13.3	$[\text{Mg}_{3.9}\text{Al}(\text{OH})_{8.8}]\text{Cl}\cdot 2.9\text{H}_2\text{O}$	8.07	47.3	256.5
Mg–Al LDH 5	25.5	6.05	14.2	$[\text{Mg}_{4.7}\text{Al}(\text{OH})_{11.4}]\text{Cl}\cdot 3.5\text{H}_2\text{O}$	8.09	29.5	224.2

† Weight loss during heating at 250°C for 2 h.

‡ Empirical formulas of the LDHs were calculated from the chemical composition considering Cl^- as the interlayer anion.

§ Surface areas were determined using the Brunauer–Emmett–Teller (BET) equation (Brunauer et al., 1938) on five-point N_2 gas adsorption isotherms.

$^\parallel$ Theoretical anion exchange capacity (TAEC) of LDHs was calculated based on the empirical formula of LDHs using the method described by Sparks (2002).

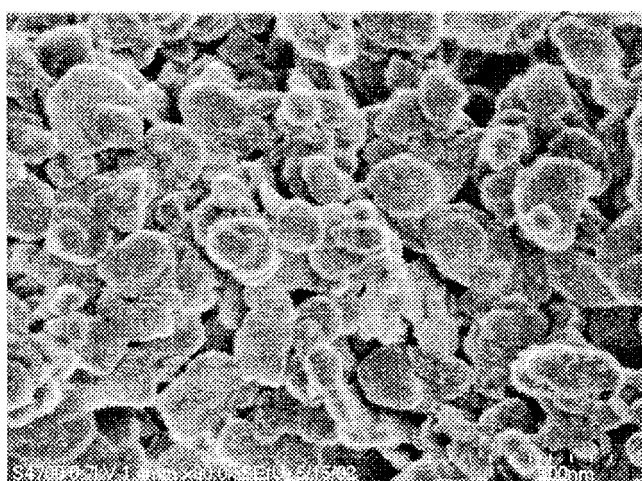
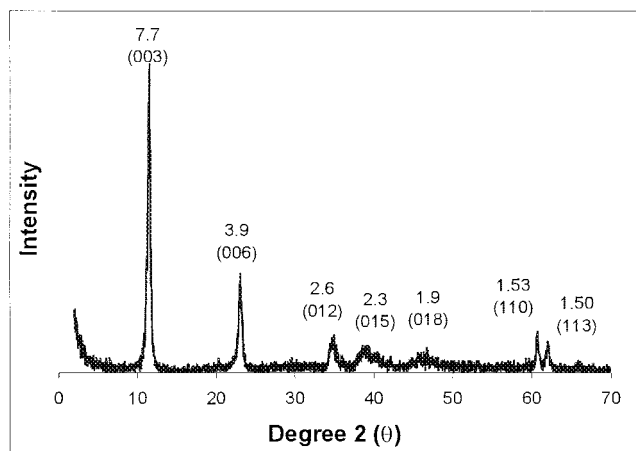


Fig. 2. X-ray diffraction pattern (top) and scanning electron microscope image (bottom) of Mg-Al layered double hydroxide (LDH) 2.

berger, 1996). Sorption results presented in Table 2 indicate that within the virus concentration range studied (5.2×10^2 to 4.5×10^5 pfu/mL), more than 99% of the MS2 was removed from aqueous solutions. At the lowest concentration tested (5.2×10^2 pfu/mL), virus particles were completely removed from solution by Mg-Al LDH 2. These results suggest that Mg-Al LDH 2 is an effective sorbent for MS2. Experiments were not done to assess the maximum LDH sorption capacity; however, the observed sorption capacity for Mg-Al LDH 2 under the conditions used showed a capacity of at least 1.51×10^{10} pfu/g. Because of its high sorption capacity, Mg-Al LDH 2 compares favorably for its po-

Table 2. Sorption of MS2 on Mg-Al layered double hydroxide (LDH) 2.

Initial MS2 concentration	Final MS2 concentration	Fraction sorbed	Relative sorption capacity†	
			pfu/mL	pfu/g
4.5×10^5	720	$99.8 \pm 0.016\%$		1.51×10^{10}
4.6×10^4	30	99.9 ± 0.015		1.53×10^9
5.4×10^3	4	99.9 ± 0.039		1.75×10^8
5.2×10^2	not detectable	100 ± 0.00		1.75×10^7

† For the experimental conditions employed, relative sorption capacity is defined as the amount of sorbed virus on unit LDH.

‡ Plaque-forming units.

§ Standard deviation.

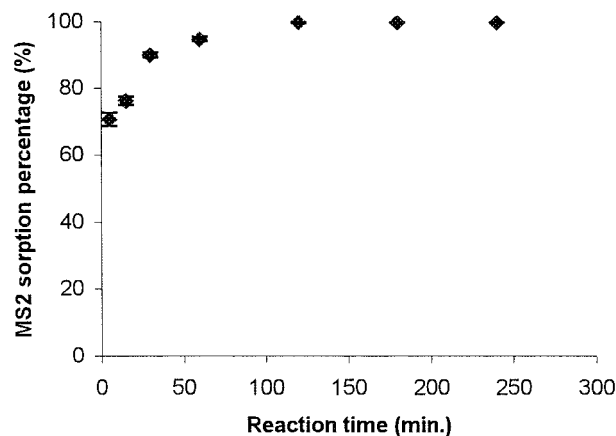


Fig. 3. Sorption kinetics for the retention of MS2 virus by Mg-Al layered double hydroxide (LDH) 2. Initial MS2 concentration was 3.6×10^5 plaque-forming units (pfu)/mL.

tential use in water treatment and drinking water purification systems with previously tested sorbent materials, such as activated carbon, metal oxide (ferric and aluminum hydrates), diatomaceous earth, sand, and insoluble polyelectrolytes.

Sorption Kinetics

One of the most important aspects for screening sorbents in terms of their potential application in point-of-use water treatment devices is reaction time. In addition to a high sorption capacity, an ideal sorbent has to rapidly remove viruses. The effect of reaction time on MS2 sorption by Mg-Al LDH 2 is shown in Fig. 3, which clearly indicates that removal of MS2 from an aqueous solution is a rapid process. A quasi-equilibrium (i.e., virus concentration in aqueous phase remained constant) was obtained within the first 1 h of sorption. During the first 5 min more than 70% of the MS2 virus was removed from the aqueous phase.

Kinetics of virus sorption on many different media have been examined previously and results indicate that virus sorption is a rapid process. For example, virus sorption to coal or cell monolayers reached equilibrium within the first 2 h of reaction time (Oza and Chaudhuri 1976; Murray and Parks, 1980). The time to reach sorption equilibrium on clay minerals was 15 min for encephalomyocarditis, poliovirus, and reovirus (Schaub and Sagik, 1975; Sobsey et al., 1980), 20 min for coliphage T2 (Carlson et al., 1968), 30 min for reovirus (Steven and Stotzky, 1983), and 45 min for bacteriophage MS2 (Stagg et al., 1977). The rapid sorption kinetics of MS2 by Mg-Al LDH 2 combined with its high sorption capacity suggests that LDHs may be ideal sorbent materials to be used in point-of-use water treatment devices. The sorption process will probably be even faster in reality where virus concentrations are generally lower than the virus challenge level of 3.5×10^5 pfu/mL used in our experiments.

Effect of Layered Double Hydroxide Type on MS2 Sorption

To compare the relative sorption capacities among LDHs with different Mg to Al ratios, batch-sorption

screening tests were conducted on all synthesized LDHs. The percentage of MS2 sorption and the relative sorption capacities on each LDH were calculated (Table 3). The results indicate that all four LDHs were effective in removing MS2 from solutions at the concentration tested (0.94×10^5 pfu/mL) but the relative sorption capacities on each individual LDH varied slightly in the following order: Mg–Al LDH 2 > Mg–Al LDH 3 > Mg–Al LDH 4 > Mg–Al LDH 5, corresponding to decreasing TAEC values of the LDHs. Although the observed differences among the treatments are too small to allow quantitative correlation between MS2 sorption and TAEC values, the sorption trend corresponding to TAEC nonetheless suggests that virus retention on LDH surfaces is probably due to electrostatic interactions.

Earlier studies suggest that LDHs possess internal and external surfaces and both are known to retain inorganic anions such as CrO_4^{2-} , SeO_4^{2-} and TeO_4^{2-} and organic surfactants like dodecylsulfate, octylbenzenesulfonate, and dodecylbenzenesulfonate (Rhee et al., 1997; Goswamee et al., 1998; Kang et al., 1999; You et al., 2001a, 2002b). However, the larger size of MS2 particles (26.0–26.6 nm) relative to the interlayer spacing (approximately 0.48 nm) of LDHs limited the possibility of virus intercalation into the LDH interlamellar structure. Consequently, sorption of viruses could only occur on the external surface of LDHs. Our results are similar to those reported by Steven and Stotzky (1984), who suggested that reovirus could not intercalate into the layer structure of montmorillonite and sorption occurred only on external surfaces.

Effect of pH on MS2 Sorption

To simulate conditions of most natural environments, sorption of MS2 to Mg–Al LDH 2 was studied at several pH values within the range of 4 to 9. The sorption percentages and relative sorption capacities of MS2 in AGW as a function of pH are listed in Table 3, which indicate that the extent of MS2 sorption by Mg–Al LDH 2 is

slightly pH dependent, decreasing with increasing pH. This is not surprising because the exterior of a MS2 virus is surrounded by a protein coat, which is composed of carboxyl and amino groups that are ionizable. As a result, variations in the net charge of the virus depend on solution pH. The isoelectric point of MS2 is 3.9 and the point zero of charge (PZC) of LDHs is approximately 10 to 11. In the pH range studied, MS2 is negatively charged and Mg–Al LDH 2 is positively charged. As pH increased, the LDH became less positively charged whereas the negative surface charge of MS2 remained approximately constant (Redman et al., 1997). Therefore, as pH increased MS2 sorption to Mg–Al LDH 2 decreased. However, MS2 sorption was high (>99%) on the LDH at all pH values tested (pH 4–9) and the less than 1% change in total sorption suggests that the pH dependence was not very significant.

Effect of Competing Anions on MS2 Sorption

Previous studies have shown that the presence of background anions in reaction systems can lead to a reduction in the sorption of anionic pollutants toward LDHs (You et al., 2001a,b, 2002a). Layered double hydroxides have greater affinities for anions with higher charge densities; for example, they can sorb more multivalent than monovalent anions (Miyata, 1983; Dutta and Puri, 1989; You et al., 2001a,b, 2002a). The results from this study show that background ions had different effects on the ability of Mg–Al LDH 2 to sorb MS2 (Table 3). The presence of SO_4^{2-} and HPO_4^{2-} decreased MS2 sorption to Mg–Al LDH 2 significantly. However, addition of NO_3^- had essentially no effect on the retention of MS2 by Mg–Al LDH 2.

These results indicate that SO_4^{2-} and HPO_4^{2-} competed effectively with MS2 for binding sites on the LDH. This indicates that LDHs should not be used in water treatment practices (such as wastewater treatment) where high concentrations of multivalent anions may exist. Rather, they would be more suited for removing water-borne viruses from contaminated drinking water

Table 3. Effects of environmental factors on MS2 sorption.

Variable	Initial MS2 concentration	Final MS2 concentration	Fraction sorbed	Relative sorption capacity [†]
	pfu/mL		%	pfu/g
Type of LDH§				
LDH 2	0.94×10^5	541	99.42 ± 0.21 ¶	0.311×10^{10}
LDH 3	0.94×10^5	1191	98.73 ± 0.43	0.309×10^{10}
LDH 4	0.94×10^5	2023	97.84 ± 0.51	0.306×10^{10}
LDH 5	0.94×10^5	3015	96.79 ± 0.29	0.303×10^{10}
pH				
4.8	3.71×10^5	130	99.96 ± 0.003	1.23×10^{10}
5.6	3.71×10^5	805	99.78 ± 0.016	1.23×10^{10}
7.5	3.71×10^5	1043	99.72 ± 0.043	1.23×10^{10}
9.0	3.71×10^5	3655	99.01 ± 0.033	1.22×10^{10}
Competing anions				
Control	0.94×10^5	541	99.42 ± 0.21	0.311×10^{10}
NO_3^-	0.94×10^5	2621	97.21 ± 1.06	0.304×10^{10}
SO_4^{2-}	0.94×10^5	0.96×10^5	-2.31 ± 5.64	NA#
HPO_4^{2-}	0.94×10^5	0.89×10^5	4.80 ± 4.82	1.50×10^8

[†] For the experimental conditions employed, relative sorption capacity is defined as the amount of sorbed virus on unit LDH.

‡ Plaque-forming units.

§ Layered double hydroxide.

¶ Standard deviation.

Not applicable.

supplies or ground water where concentrations of anions are much lower than the tested concentration in this study (Sparks, 2002).

Some surface interactions, including electrostatic sorption, van der Waals forces, covalent–ionic interactions, ion-bridge, hydrogen bonding, and hydrophobic reactions, have been reported as the mechanisms for virus attachment on solid particles (Steven and Stotzky, 1983; Gerba, 1984; Chu et al., 2000). Viruses are charged colloid particles and their sorption to solid surfaces depends upon the nature of the sorbent and upon ionic composition and pH of the medium in which they are suspended. Results from this study suggest that sorption of MS2 on LDHs was probably due to electrostatic attraction because (i) sorption of MS2 on LDHs decreased with decreasing LDH TAEC, (ii) bivalent competing anions decreased the retention of MS2 on LDHs significantly, (iii) sorption of MS2 decreased with increasing solution pH, (iv) there was no significant relationship between MS2 sorption and total LDH surface areas, and (v) there was no organic matter involved in the experiments, so hydrophobic interactions were unlikely.

Sorption of MS2 in the Presence of ϕ X174

More than one type of virus is likely to be present in microbial contaminated environments. Therefore, we conducted experiments to test the effect of an additional bacteriophage, ϕ X174, on the sorption of MS2 by Mg–Al LDH 2. ϕ X174 is a spherical single-stranded DNA virus about 23 nm in diameter with a isoelectric point of 6.6 (Ackermann and Dubow, 1987, p. 190–191). Under the experimental pH of 7.5, ϕ X174 is negatively charged and expected to sorb on positively charged LDH surfaces, competing with MS2. A competitive sorption experiment was conducted at two ϕ X174 concentrations (1.2×10^4 and 1.1×10^3 pfu/mL) while keeping a constant MS2 concentration (9.4×10^4 pfu/mL). Table 4 indicates that competition by ϕ X174 only caused a slight decrease in the retention of MS2 by Mg–Al LDH 2, suggesting that there was minor competitive sorption between MS2 and ϕ X174 on LDH surfaces. Table 4 also suggests that Mg–Al LDH 2 has a high sorption capacity for ϕ X174 as well.

Column Experiments

Batch sorption experiments provide general information on virus retention on solid surfaces. Column transport experiments can provide better assessment on sorption under flow conditions. In this study, we used Mg–Al LDH 2-coated sand in a column transport experiment

Table 4. Competitive virus sorption on Mg–Al layered double hydroxide (LDH) 2.

Virus concentration	MS2 fraction sorbed	ϕ X174 fraction sorbed
	%	
MS2 (9.4×10^4 pfu [†] /mL)		
ϕ X174 (1.1×10^3 pfu/mL)	98.3 \pm 0.60 [‡]	99.4 \pm 0.22
ϕ X174 (1.2×10^4 pfu/mL)	97.9 \pm 0.49	94.5 \pm 0.31

[†] Plaque-forming units.

[‡] Standard deviation.

to assess the efficiency of LDH 2 as a filtration–sorption medium to remove MS2 under continuous flow conditions. Compared with the oxide-removed sand surface, the SEM image of Mg–Al LDH 2-coated sand surface showed well-defined hexagonal plate-shaped Mg–Al LDH 2 crystals, suggesting that Mg–Al LDH 2 was successfully coated on the oxide-removed sand surface (Fig. 4). Breakthrough curves of Br[−] from both oxide-removed sand and Mg–Al LDH 2-coated sand columns indicate that the hydrodynamic conditions in both columns were similar (Fig. 5). MS2 showed a rapid rise in the outflow concentration within the first 3 pore volumes (PV) from the oxide-removed sand column and reached a plateau at approximately 5 PV with a final relative concentration (C/C_0) of approximately 0.85. The breakthrough curve of MS2 in Mg–Al LDH 2-coated sand was significantly different from that of the oxide-removed sand column results. No viruses were detected in the effluent samples for the entire duration of the experiment (approximately 50 PV), indicating that Mg–Al LDH 2-coated sand was highly effective in MS2 sorption. Results of the column experiments agree well with the batch experiments and suggest that LDH mate-

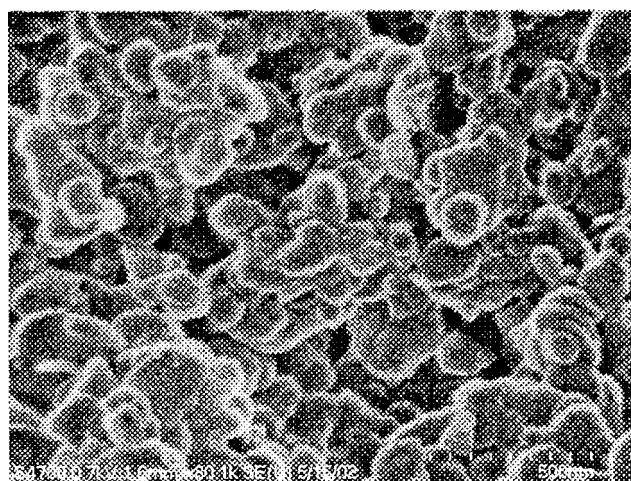
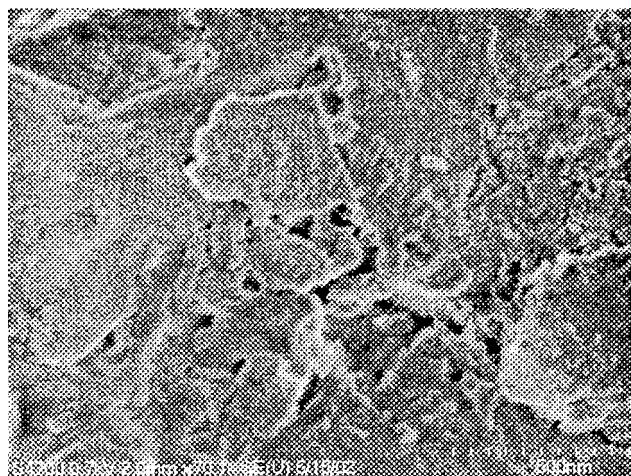


Fig. 4. Scanning electron microscope images of oxide-removed sand (top) and Mg–Al layered double hydroxide (LDH) 2-coated sand (bottom).

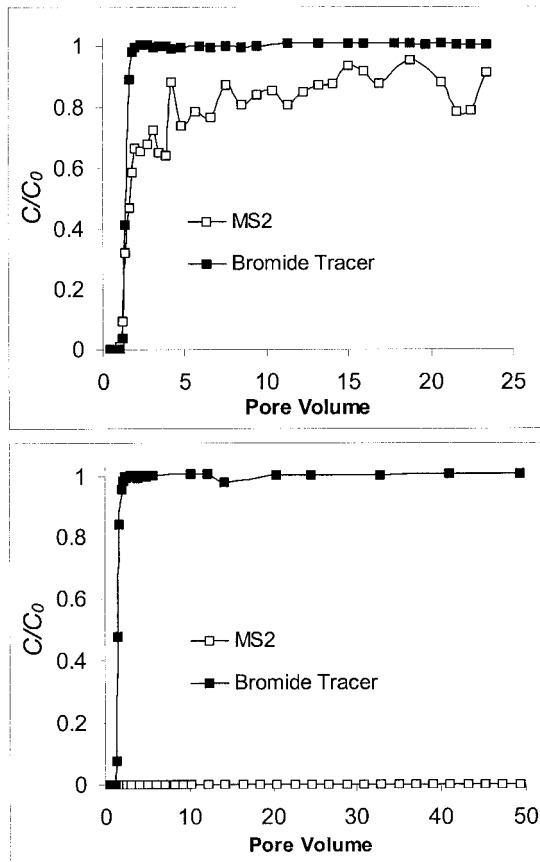


Fig. 5. Virus breakthrough curves from columns packed with oxide-removed sand (top) and Mg–Al layered double hydroxide (LDH) 2-coated sand (bottom).

rials may be well suited in point-of-use devices as filtration or sorption media to remove water-borne viruses.

Mass recoveries, calculated based on results of beef extract elution, were 77.9 and 114.5% for the oxide-removed sand column and Mg–Al LDH 2-coated sand column, respectively, indicating that the virus particles attached on Mg–Al LDH 2 were still viable during the attachment–detachment process. The high mass recovery from the Mg–Al LDH 2-coated sand column suggests that removal of viruses by Mg–Al LDH 2 was due to sorption of MS2 on Mg–Al LDH 2 surface rather than inactivation.

CONCLUSIONS

Microbiological contamination of drinking water remains to be one of the greatest challenges in public health risk management. The development of new technologies that can improve the efficiency of currently available water treatment methods is urgently needed. Through batch and column experiments, we demonstrated that LDHs could potentially be used as sorbent materials for removing water-borne viruses in water treatment facilities and at drinking water taps. Our study also showed that sorption of viruses on LDHs was a rapid process and was not affected significantly by pH in the range between 4 and 9. The lack of pH dependence suggests that LDHs would be effective sorbents under

most environmental pH conditions. However, the viral removal efficiency of LDHs would be significantly reduced in the presence of bivalent anions SO_4^{2-} and HPO_4^{2-} , because our results indicate that those anions compete strongly with viruses for sorption on LDHs. Therefore, LDHs may not be suitable for use in treatment systems where the concentration of multivalent anions is high, which might be considered a disadvantage for the potential application of LDHs. We also found that the retention of viruses by LDHs was due to sorption that was reversible under high pH and high ionic strength (beef extract) conditions rather than inactivation, which would be the preferred process.

The current study represents the very first attempt to evaluate LDHs as potential sorbents for viruses. The pitfalls identified along with the more practical questions, such as cost benefits of using LDHs versus other types of sorbents, the reusability of LDHs, and regeneration of used LDHs, need to be addressed to determine whether the use of LDHs will be a viable technology for water treatment purposes.

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