Removal of bacteria and viruses from waters using layered double hydroxide nanocomposites
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Abstract

We have identified synthetic layered double hydroxides (LDH) nanocomposites as an effective group of material for removing bacteria and viruses from water. In this study, LDH nanocomposites were synthesized and tested for removing biological contaminants. LDH was used to remove MS2 and \textit{f}X174 (indicator viruses), and \textit{Escherichia coli} (an indicator bacterium) from synthetic groundwater and to remove mixed communities of heterotrophic bacteria from raw river water. Our results indicate that LDH composed of magnesium–aluminium or zinc–aluminium has a viral and bacterial adsorption efficiency \textgreater99\% at viral concentrations between \(5.9 \times 10^6\) and \(9.1 \times 10^6\) plaque forming units (pfu)/L and bacterial concentrations between \(1.6 \times 10^{10}\) and \(2.6 \times 10^{10}\) colony forming units (cfu)/L when exposed to LDH in a slurry suspension system. Adsorption densities of viruses and bacteria to LDH in suspension ranged from \(1.4 \times 10^{10}\) to \(2.1 \times 10^{10}\) pfu/kg LDH and \(3.2 \times 10^{13}\)–\(5.2 \times 10^{13}\) cfu/kg LDH, respectively. We also tested the efficiency of LDH in removing heterotrophic bacteria from raw river water. While removal efficiencies were still high (87–99\%), the adsorption capacities of the two kinds of LDH were 4–5 orders of magnitude lower than when exposed to synthetic groundwater, depending on if the LDH was in suspension or a packed column, respectively.

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1. Introduction

Layered double hydroxides (LDH) nanocomposites are a group of anionic clay-like materials with unique layered structures. The general formula of LDH is \([\text{M}_2\text{X}_x\text{OH}_2\text{]}_{\text{n}}\), where \(\text{M}^{2+}\) and \(\text{M}^{3+}\) are bivalent and trivalent cations, respectively, in octahedral positions and \(\text{X}^{m-}\) is an anion positioned between the interlayers. Synthesized LDH is highly crystalline and composed of well-defined hexagonal plate-shape crystals, with the diameter being approximately 100 nm. Due to the substitution of trivalent cation \(\text{Al}^{3+}\) by divalent cations \(\text{Zn}^{2+}\) and \(\text{Mg}^{2+}\) in octahedral structure, the LDH nanoparticles have positively charged sheets. Layered double hydroxides are rare in nature, but can be synthesized by coprecipitating bivalent and trivalent metal salts with a base under controlled conditions [1–5].

Pathogenic bioagents, such as bacteria and viruses, exist ubiquitously in water systems. Under natural pH conditions, the polypeptides of bacteria and viruses are composed of weakly acidic and basic groups (e.g., carboxyl and amino moieties), which are ionizable, presenting a negative surface charge. In addition, the configurations of bacterial and viral structures present a negative charge on their surface [6–10].

Viruses have been reported to adsorb to LDH-coated sand due to the high anion exchange capacities and surface area of LDH [5], which makes LDH a promising material for removing biological molecules from water. Some commonly used filtration systems, including activated carbon, ion exchange resins, and synthetic fabrics, effectively remove organic compounds and certain cationic metals; however, these sorbents possess neutral or negatively charged surfaces...
and, therefore, cannot specifically adsorb negatively charged bioagents. Nanoscience and nanotechnology have grown exponentially over the last decade in various areas, including waste treatment and contaminant remediation. The unique positive charge of LDH and its selective affinity for negatively charged molecules demonstrates a promising technology for removing bacteria and viruses from drinking water [11].

The purpose of this study was to evaluate the removal efficiency of viruses and bacteria from synthetic and raw water using magnesium–aluminium (Mg–Al) and zinc–aluminium (Zn–Al) LDH nanocomposites in slurry-suspension and packed-column experiments.

2. Methods and materials

2.1. Preparation of Mg–Al and Zn–Al LDH

The Mg–Al and Zn–Al LDH nanocomposites were prepared according to You et al. [12,13] at a Mg:Al or Zn:Al molar ratio of 2:1. Mg–Al and Zn–Al LDH was prepared by mixing MgCl$_2$·6H$_2$O or ZnCl$_2$ with AlCl$_3$·6H$_2$O (all salts from Aldrich, St. Louis, MO) in deionized water (total metal concentration equal to 1.0 M) at 25°C. The pH of the solution was then elevated using NaOH (Aldrich, St. Louis, MO), which caused metal coprecipitation (i.e., LDH material) until the solution reached a pH of 10. The mixture was then transferred to bottles and heated to 80°C for 24 h. Next, the precipitates were washed with deionized water until no chloride was present, dried at 65°C, ground, and stored at room temperature (20–22°C).

Characterization of the synthesized material was conducted by using X-ray diffraction and reported separately by You et al. [12]. A well-ordered crystalline structure was observed with chloride as the anion in the interlayer of the synthetic LDH. Additional characterization by ICP indicated a Mg/Al mole ratio of 1.9.

2.2. Virus and bacteria assays

The bacteriophages MS2 and φX174 from the American Type Culture Collection (ATCC 15597-b1 and ATCC 13706-b1, respectively) were grown on Escherichia coli (ATCC 15597 and 13706, respectively) by the agar overlay method described in [14]. The enumeration of the bacteriophages was performed by the plaque assay method described in [5,14]. A 1-mL portion of the E. coli culture and a diluted (1–1000-fold) virus solution were added to trypticase soy agar (TSA) and poured into petri dishes to solidify. The TSA plates were then incubated at 37°C for 16 h.

Bacterial populations were quantified using the viable heterotrophic bacteria plate count method described in [15]. For each selected dilution, a 0.01-mL sample was transferred and spread on a TSA plate and incubated for one week at 35°C.

2.3. LDH slurry suspension experiments

Sorption experiments were conducted to investigate adsorption of viruses and bacteria to two types of LDH (Mg–Al and Zn–Al) in suspension. Stock solutions of pure strains of MS2, φX174, and E. coli were diluted (1–1000-fold) with synthetic groundwater (containing 0.075 mM CaCl$_2$, 0.082 mM MgCl$_2$, 0.051 mM KCl, and 1.5 mM NaHCO$_3$, pH 7.10) to a final solution volume of 29 mL in 50-mL centrifuge tubes. Ionic strength of this solution is 1.96 mM. The concentration of LDH in suspension in these experiments ranged from 431 to 1666 mg/L. The tubes were shaken with an end-over-end shaker at 25 rpm for 3 h at 4–7°C and centrifuged at 9000 × g for 15 min. Final concentrations of viruses and bacteria were quantified using plate count assays.

Raw water from the Laramie River (Laramie, WY, pH 7.20, ionic strength 7.5 mM) was used in similar suspension experiments where 30 mL of water was treated with 1000 mg/L of each type of LDH for 3 h in suspension.

2.4. LDH column experiment

Recognizing that a slurry suspension may not be the most feasible method for treating large volumes of water, we conducted flow-through column experiments to investigate the adsorption capacity of bacteria on LDH in packed columns. Plastic, 10-mL columns (2-cm OD × 10-cm height) were filled with 5 g of LDH (Mg–Al or Zn–Al, no packing) and a total of 200 mL of Laramie River water was pumped through the column at 0.82 mL/min. The effluent was collected and the bacteria were quantified using plate count assays.

3. Results

3.1. Synthetic ground water in suspension

On average, when exposed to LDH in a suspension slurry, 99.0 ± 0.2% of E. coli (ATCC 13706) and 99.1 ± 0.5% E. coli (ATCC 15597) were removed at adsorption densities of $3.2 \times 10^{13} \pm 7.1 \times 10^{10}$ and $5.2 \times 10^{13} \pm 2.8 \times 10^{11}$ cfu/kg LDH, respectively. Additionally, an average of 99.4 ± 0.6% of φX174 and 99.8 ± 0.1% of MS2 were removed from water with average adsorption densities of $1.4 \times 10^{0} \pm 8.0 \times 10^{7}$ and $2.1 \times 10^{0} \pm 1.6 \times 10^{7}$ pfu/kg LDH, respectively. There was no difference in adsorption efficiency of viruses or bacteria between LDH composed of Mg–Al or Zn–Al (Fig. 1).

3.2. River water in suspension

The Mg–Al and Zn–Al LDH displayed high removal efficiencies of heterotrophic bacteria from river water, decreasing cfu concentrations by 98.1 ± 0.3% and 98.4 ± 0.5%, respectively. The adsorption densities of mixed heterotrophic bacteria in raw river water on the
Mg–Al and Zn–Al LDH was about one order of magnitude less than when exposed to monocultures in synthetic groundwater. The adsorption densities of the Mg–Al and Zn–Al LDH were 3.4 × 10^9 and 3.4 × 10^7, respectively.

3.3. Column filtration of river water

The adsorption efficiencies of heterotrophic bacteria from raw river water to LDH in flow-through columns were presented in Fig. 2. Since bacteria concentrations vary from different sources, we normalized the unit in X-axis to be “bacteria number (in 10^5)/g LDH”, which was converted as X = bacterial concentration × water volume/10,000. The results are similar to those measured for river water in the LDH suspension, however, the adsorption capacities were about one order of magnitude lower.

Adsorption efficiency of bacteria onto Mg–Al LDH decreased from 99.0 ± 1.0% to 86.9 ± 2.6% and adsorption efficiency onto Zn–Al LDH decreased from 99.5 ± 0.5% to 93.8 ± 0.9% after 200 mL of river water had passed through each column. The maximum adsorption capacities of bacteria on the Mg–Al Zn–Al and LDH measured during this experiment were 6.7 × 10^8 and 7.2 × 10^8 cfu/kg, respectively. The Zn–Al LDH displayed slightly higher removal efficiency than the Mg–Al LDH in the column exposures (Fig. 2).

4. Discussion

The long list of pathogenic microbes and other biological molecules such as endocrine-active compounds currently identified (e.g., Ref. [19]) will only grow longer as new and existing chemicals are used with higher frequency. These compounds expose significant threat to human health. Therefore, an effective and specific treatment process must be used to reduce the concentrations of these biological agents. The unique structure of LDH makes it an ideal candidate for such application. Results indicate that LDH can adsorb >99% of bacteria and viruses, used in this study, from a synthetic groundwater when exposed in suspension. The loading capacities for bacteria and viruses are about 10^{13} and 10^{10} pfu/kg, respectively. Compared with other available sorption material, such as carbon-based media, clay minerals, metal oxides/hydroxides and activated carbon, LDH demonstrates substantially higher removal efficiency and capacity. For example, Oza and Chaudhuri [16–18] found that bituminous coal could only remove 70% of bacteriophages (T4 and MS2) from an aqueous solution.

The column and suspension experiments demonstrated that LDH could potentially be used as a sorbent material...
to remove bacteria from untreated water, such as raw river water. Using LDH to polish the biological agents removal from the treated municipal wastewater effluent will significantly benefit the community. We anticipate this research leading to an alternative treatment approach for municipal Waste Water Treatment Facilities (WWTFs) as well as treatment facilities associated other sources of biological agents.

However, a few points need to be addressed in developing an applicable LDH-based technology for water treatment. First, anion competition for binding sites may decrease overall sorption capacities of LDH. For example, CO$_3^{2-}$ and HPO$_4^{2-}$ may be sorbed on the LDH surface and saturate the sorption sites, resulting in a decrease in sorption capacity [5]. This may explain why the sorption capacity of bacteria on LDH in suspension in river water ($\sim 10^7$ cfu/kg LDH) was four orders of magnitude lower than in synthetic groundwater ($\sim 10^{13}$ cfu/kg), which presumably contains fewer competing anion species than raw river water. Furthermore, a suitable filtration matrix that can support the LDH must be developed. Currently, it seems that suspension slurries are not feasible for large treatment volumes and although packed columns can efficiently remove biological molecules, this type of filtration system reduces the adsorption capacity of the LDH by at least one order of magnitude compared to suspension exposures. Efforts are currently attempted to modify the structures of LDH to make the material feasible for filtration applications. We have successfully polymerized LDH and made granular form of LDH–polymer mixture [11]; however, the adsorption efficiencies of such a product remains to be determined.

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