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Short communication

Removal of endocrine active compounds using layered double hydroxide material

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ABSTRACT

A granular form of a layered double hydroxide (LDH) material was used as an anionic adsorbent in packed column and slurry experiments to remove endocrine active compounds (EACs) from river water downstream from wastewater treatment plants and from laboratory water spiked with 17β-estradiol (E2). The estrogenic activity of the samples was estimated using the biological yeast estrogen screen (YES) assay and the E2 concentrations were analyzed using radioimmunoassay techniques. The LDH in a packed column significantly ($p < 0.05$) decreased the estrogenic activity of the river water from 519 to 387 ng E2 equiv./L after one pass through the column. The LDH packed column reduced the E2 concentration in a different river water sample from 12 ng/L to below detection limit (1.8 ng/L) with minimal retention time. Finally, LDH in a slurry treatment reduced the E2 concentration in water from 317 ng/L to below detection limit. The results of these experiments suggests that LDH may be used to treat waste or drinking water for estrogenic endocrine disrupting components (EDCs) such as E2 and possibly other anionic compounds that affect the endocrine system.

1. Introduction

Endocrine active compounds (EACs) are natural or synthetic compounds that adversely affect the endocrine system of many organisms ranging from nematodes to fish to polar bears [1–3]. Many natural and synthetic compounds present in watersheds in the USA (and likely other countries) can cause adverse effects on the endocrine systems of various organisms [4]. These compounds include natural and synthetic hormones, herbicides, pesticides, pharmaceuticals, and other personal care products. Therefore, there are countless point and non-point sources of EACs including municipal wastewater treatment plants or agricultural operations that could potentially contaminate water supplies.

Two common EACs found in the waterways are the biogenic hormone 17β-estradiol (E2; CASRN 50-28-2; FW 272.3864) and the synthetic hormone 17α-ethynylestradiol (EE2; CASRN 57-63-6; FW 296.4084), which have both been identified downstream from municipal or agricultural waste treatment plants in concentrations as high as 200 and 831 ng/L, respectively [5]. Very low concentrations (ng/L range) of E2 and (or) EE2 have been shown to affect the reproductive physiology and (or) behavior of many organisms. For instance, extremely low concentrations of E2 (21 ng/L) and EE2 (3.0 ng/L) are sufficient to cause significant induction of vitellogenin in male zebrafish (*Danio rerio* [6]). Furthermore, Martinović et al. [7] found that wastewater treatment plant (WWTP) effluent from a treatment plant in St. Paul, MN, USA and waterborne E2 (~50 ng/L) prevented male fathead minnows (*Pimphales promelas*) from reproducing when they had to compete with non-exposed males. Woodling et al. [2] found multiple adverse reproductive maladies in white sucker (*Catostomus commersoni*) populations downstream from a WWTP in Boulder, CO, USA, including decreases in male:female sex ratios, gonad deformities, and intersex fish. Similarly, high numbers of intersex fish (male fish with testicular oocytes) have also recently been reported in the Potomac River near Washington, DC, USA. Although the cause of these intersex fish is still under investigation the presence of endocrine disrupting components (EDCs) such as estrogen hormones in the water has been suspected as a cause [8]. Finally, mixtures of near detection limit concentrations of multiple EACs (including E2, EE2, 4-*tert*-nonylphenol, 4-*tert*-octylphenol, and bisphenol) can significantly affect the reproductive performance of fathead minnows [9].

In a previous study, we determined that Mg–Al layered double hydroxide (LDH) material could effectively remove bacteria and viruses from natural water sources due to the unique structure and surface charge of this material, which gives LDH a high capacity

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to adsorb negatively charged molecules [10]. The purpose of this research was to determine if a granular form of LDH prepared as described in Jin et al. [10] could also effectively remove anionic EACs through adsorption from river water collected downstream from WWTPs and reverse osmosis (RO) water spiked with E2 in the laboratory. Our overall research objective is to develop LDH as a possible water purification tool for removing several contaminates from a growing list of EACs, of which the effects on human health remain largely unknown while the effects on other animals and insects are becoming more disturbingly apparent.

2. Methods

Water samples were collected downstream from municipal WWTP in Wyoming, USA during August 2006 and exposed to a Mg–Al LDH material prepared as described in Jin et al. [10]. Due to the semi-quantitative nature of the yeast estrogen screen (YES) assay, similar experiments were repeated using a more quantitative analytical technique (radioimmunoassay) after initial data generated with the YES assay indicated exposure to LDH decreased the estrogen activity of the contaminated water. The experiments described below are grouped according to the analytical test method used to determine either the overall estrogen activity (YES assay experiment) or the 17β-estradiol (E2) concentration (radioimmunoassay experiments).

2.1. YES assay experiment

Water was collected from the Tongue River 800 m downstream from the WWTP in Sheridan, WY, USA, filtered by an 0.45 μm filter, stored in a 1-L ethanol-washed amber glass bottle and transported on ice to Western Research Institute in Laramie, WY, USA. Five grams LDH was packed into four 20-mL sterile plastic syringes and river water was poured into the syringes and allowed to drip through at a rate of approximately 1 mL/min. The estrogen activity of four pre- and post-filtration samples was estimated by using a modified version [11,12] of the YES assay developed and first described by Routledge and Sumpter [13]. We conducted statistical comparisons of the estrogen activity (as E2) of the LDH-filtered and unfiltered samples with ANOVA ($\alpha = 0.05$) using Minitab™ Version 13.31 (Minitab Statistical Software, Minitab Inc.).

2.2. Radioimmunoassay experiments

Water was collected from the discharge point at the WWTP in Laramie, WY, USA and approximately 400 m downstream of the discharge point from this WWTP in the Laramie River on 2 August 2007. This water was collected in ethanol-washed glass beakers, coarsely filtered through a small column packed with glass wool and used in packed LDH column tests immediately. An additional water sample was collected from the same point on the Laramie River on 6 August 2007 and treated as the previous samples except it was used in a series of slurry mixing tests with LDH.

2.2.1. Column test

Five grams of LDH was packed into two 20-mL plastic syringes and test water was poured into the syringes and allowed to drip through at a rate of approximately 1 mL/min as in the YES assay experiment. Two-milliliter samples were collected prior to addition to the syringe and at breakthrough volumes of 2, 10, 50, 100, 200, 500, 750, and 1000 mL. All samples were collected in ethanol-washed glass tubes and frozen for analysis of E2. Additional 0.5-mL samples were collected at the same intervals in plastic microcentrifuge tubes and stored at 4 °C for analysis of major anions.

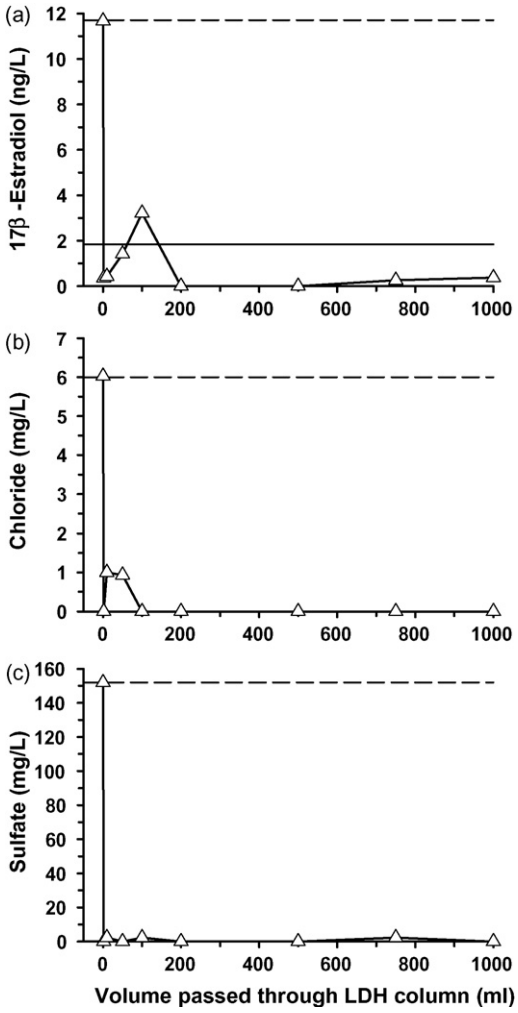

Fig. 1. Changes in (a) 17β-estradiol and (b and c) anion concentrations in Laramie River water collected downstream of the Laramie WWTP filtered through 5 g of LDH in a column. Horizontal dashed line indicates baseline concentrations and horizontal solid line indicates 17β-estradiol detection limit.

2.2.2. Slurry test

Due to the low concentrations of E2 in the Laramie River water and WWTP effluent, a second set of tests was conducted using deionized water spiked with E2 (Steraloids, Inc., New Port, RI, USA, Cat. #EO950-000). This test water was mixed using a concentrated solution of 1000 mg E2/L acetone that was added to the water to make a stock solution of E2 at 1 mg/L, which was diluted to a nominal value of 300 ng E2/L (1101.3 pmol/L). Fifty milliliters of this spiked water was added to three ethanol-washed glass flasks containing 0.1 g of Mg–Al LDH. One flask containing spiked water was placed on a shaker incubator (200 rpm) at 25, 35, or 45 °C for a total of 3 h. Water samples were collected and stored as described for the column test prior to addition to each flask and after 2, 5, 10, 25, 50, 100, and 180 min of shaking.

2.2.3. Chemical analyses

Concentrations of E2 were analyzed at the Colorado State University Reproductive Endocrinology Laboratory (Fort Collins, CO, USA) by using radioimmunoassay techniques described under the Endocrinology Laboratory's standard operation procedure SOP-RIA-E2-6-001-00. The method detection limit for these analyses was 1.84 ng E2/L. Major anions including bromide (Br^-), chloride (Cl^-), fluoride (F^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulfate

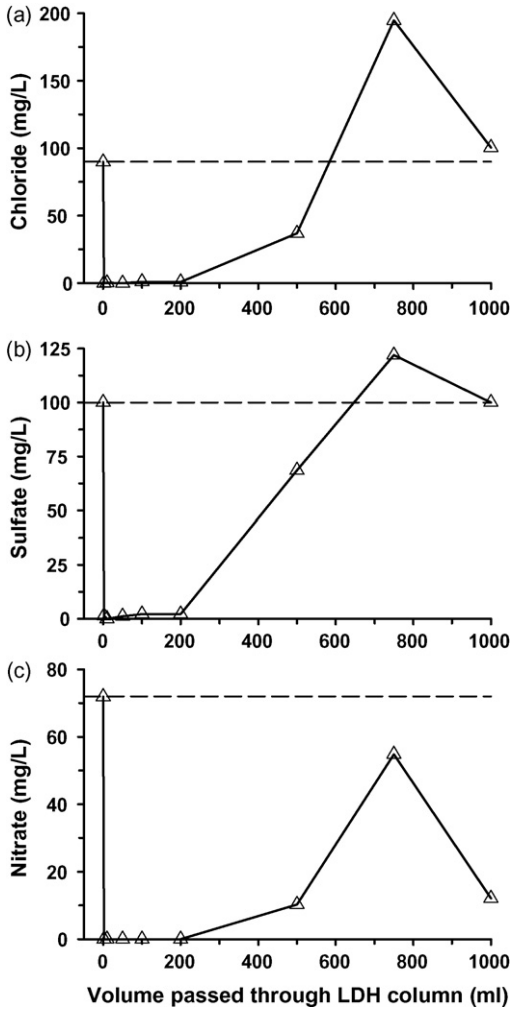

Fig. 2. Changes in anion concentrations in Laramie WWTP effluent filtered through 5 g of LDH in a column. Baseline concentrations are shown with a dashed horizontal line.

(SO_4^{2-}) were analyzed on a DIONEX DX-100 Ion Chromatograph equipped with a 4 mm × 250 mm IonPac AS14 anion exchange column.

3. Results and discussion

3.1. YES experiment

The average ($n = 4$; ±S.D.) estrogen activity of river water collected below the Sheridan WWTP in the Tongue River decreased significantly ($p < 0.05$) from 519 (±9) to 387 (±61) ng E2 equiv./L when filtered through LDH columns.

3.2. Column test

Concentrations of E2 in effluent from the Laramie WWTP were below the method detection limit (MDL; 1.84 ng/L) at 1.67 ng/L; however, the concentration of E2 in these samples showed a declining trend when passed through the LDH column and concentrations decreased to as low as 0.3 ng/L during the test. These data are not shown because all concentrations were below the MDL. The concentration of E2 in the Laramie River downstream from the WWTP was 11.7 ng/L and the concentration of E2 in the column effluent decreased to below the MDL within the first 2 mL passed through the column and remained below the MDL at each subsequent sample taken except at the 100-mL sample interval when the concentration of E2 in the column effluent increased to 3.2 ng/L but then returned to below detection limit in the subsequent samples (Fig. 1a). The detected 3.2 ng/L was presumably attributed to the sampling error during the test.

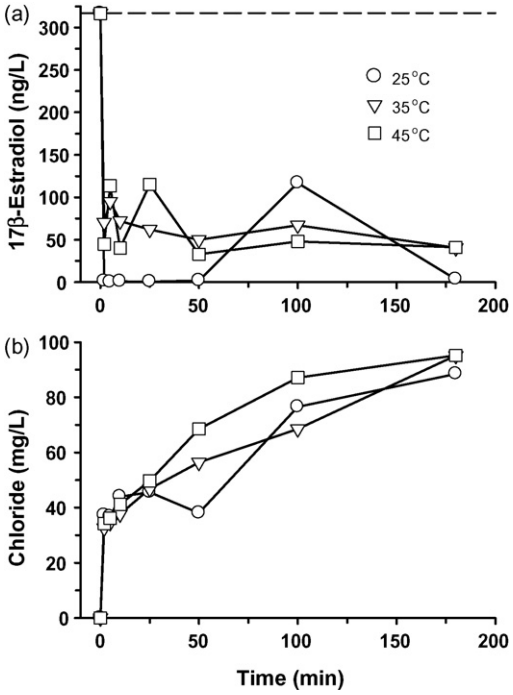

Fig. 3. Changes in (a) 17β-estradiol and (b) chloride concentrations over time in 50 mL of water spiked with 317 ng 17β-estradiol/L (dashed horizontal line) exposed to 0.1 g of LDH in a slurry at varying temperatures.

Concentrations of chloride and sulfate decreased substantially after the first 2–100 mL were passed through the column (Fig. 1b and c, respectively). Nitrate was not detected in the initial river water samples used in this test. The concentrations of chloride, sulfate and nitrate in the WWTP effluent all decreased immediately to non-detect, and eluted out after 200 mL of effluent had been passed through the LDH column (Fig. 2a–c, respectively), suggesting a non-specific affinity of LDH to these anions. Eluted chloride exceeded the starting concentration, probably due to the residual chloride contained in the LDH during its synthesis [10]. This "catch-and-release" pattern of anion sorption by LDH indicates possible sorption competition between anions and EDCs.

3.3. Slurry test

Changes in temperature did not significantly influence E2 adsorption to LDH. The concentration of E2 decreased rapidly from 316.7 ng/L in the initial spiked sample to below MDL, 70.6, and 44.7 ng/L in the 25, 35, and 45 °C treatments, respectively within the first 2 min of shaking. The concentration of E2 in the 25 °C treatment increased to 116.9 ng/L after 100 min but then decreased to 3.3 ng/L at 180 min while the concentrations of E2 in the 35 and 45 °C treatments exhibited less variability throughout the test and were 40.4 and 41.1 ng/L, respectively, at the end of the test (Fig. 3a). Chloride concentrations steadily increased from below detection limit to between 80 and 100 mg/L in all three test temperatures (Fig. 3b). This is consistent with the releasing of residual chloride from LDH material as observed in the previous tests.

The results of our preliminary investigations using river water containing E2 and clean water spiked with E2 indicate LDH can

remove E2 and decrease overall concentrations from toxicologically significant concentrations (e.g. 11–300 ng/L) to below detection limit within minutes. Furthermore, the results of the YES assay experiment indicate the overall estrogen activity of the contaminated river water decreases significantly when filtered with LDH, which suggests that LDH can effectively remove endocrine active chemicals other than and in addition to E2.

As the U.S. Environmental Protection Agency and its counterpart organizations in other countries begin to monitor and regulate many EDCs and private citizens begin to take an interest in assuring these chemicals are not in their drinking water, methods for removal of EDCs need to be developed and (or) improved. The ease of production and relative low cost of using LDH as a filtration substrate to remove EDCs warrants further investigation into the efficiency of removing EDCs other than E2 with LDH packed columns or slurry techniques.

4. Conclusions

The ability of LDH to remove anionic molecules including bacteria and viruses [10], EDCs (results from this paper), and metalloids including negatively charged arsenic and selenium species (e.g. [14]) suggests that this material may be an ideal water purification tool for both industrialized and third-world communities. More research is currently being conducted at Western Research Institute to determine loading capacities of various contaminants on LDH and optimal material production protocols.

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