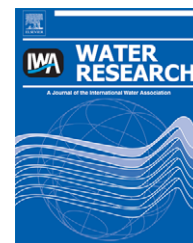


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Influence of organic matter on the transport of *Cryptosporidium parvum* oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium

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ABSTRACT

To assess the effect of organic matter on the transport of *Cryptosporidium parvum* oocysts in a geochemically heterogeneous saturated porous medium, we measured the breakthrough and collision efficiencies of oocysts as a function of dissolved organic matter concentration in a flow-through column containing ferric oxyhydroxide-coated sand. We characterized the surface properties of the oocysts and ferric oxyhydroxide-coated sand using micro-electrophoresis and streaming potential, respectively, and the amount of organic matter adsorbed on the ferric oxyhydroxide-coated sand as a function of the concentration of dissolved organic matter (a fulvic acid isolated from Florida Everglades water). The dissolved organic matter had no significant effect on the zeta potential of the oocysts. Low concentrations of dissolved organic matter were responsible for reversing the charge of the ferric oxyhydroxide-coated sand surface from positive to negative. The charge reversal and accumulation of negative charge on the ferric oxyhydroxide-coated sand led to increases in oocyst breakthrough and decreases in oocyst collision efficiency with increasing dissolved organic matter concentration. The increase in dissolved organic matter concentration from 0 to 20 mg L⁻¹ resulted in a two-fold decrease in the collision efficiency.

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1. Introduction

Contamination of drinking water by pathogenic microbes – viruses, bacteria, and protozoa – is considered as one of the most important water supply problems of our day. Current concern is focused on *Cryptosporidium parvum*, a protozoan parasite that moves from host to host as an oocyst (Carey et al., 2004). An outbreak of *Cryptosporidium parvum* caused the death of over 100 citizens of Milwaukee, Wisconsin, in 1993 (MacKenzie et al., 1994). Water utilities are increasingly using bank filtration to remove microbes from surface water supplies and to reduce the need for disinfection (Tufenkji

et al., 2002; Gollnitz et al., 2003). Bank filtration uses alluvial aquifer sediments to filter microbes from river water. The effectiveness of microbe removal by bank filtration removal depends on the characteristics of the pumping-induced groundwater flow, the alluvial sediments, and the solution chemistry. Removal can be difficult to predict because of physical and geochemical heterogeneity of the alluvial sediments and the pore waters.

One form of geochemical heterogeneity in alluvial aquifers that affects microbe removal is the nature and abundance of organic matter. Organic matter is present in the sediments as lignin, proteins, kerogen, and black carbon derived from

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terrestrial plants in discrete phases and mineral coatings at concentrations ranging up to a few percent. In the pore waters, organic matter is present as dissolved humic substances and organic acids of lower molecular weight at concentrations up to a few milligrams of carbon per liter. During bank filtration, the nature and abundance of dissolved organic matter is altered by a variety of biological processes and sorption (Miettinen et al., 1994; Cosovic et al., 1996; Grünheid et al., 2005).

In aquifer sediments, minerals like ferric and aluminum oxyhydroxides and clay edges are typically coated by organic matter (Amelung et al., 2002; Wagai et al., 2009). These minerals often form patchy coatings and interstitial aggregates on primary mineral grains (Ryan and Gschwend, 1992; Coston et al., 1995; Penn et al., 2003). Ferric and aluminum oxyhydroxides and clay edges are amphoteric minerals with relatively high points of zero charge (pH_{pzc}). Typical groundwater pH values are below their pH_{pzc} values; therefore, these minerals adsorb protons and acquire positive surface charge. At typical groundwater pH values, most pathogenic microbes – viruses, bacteria, protozoa – are negatively-charged. Positively-charged surfaces effectively remove negatively-charged colloids (Song et al., 1994; Johnson et al., 1996) and microbes (Scholl and Harvey, 1992; Mills et al., 1994; Zhuang and Jin, 2003b; Abudalo et al., 2005; Foppen and Schijven, 2005; Hijnen et al., 2005; Kim et al., 2008) from passing pore waters. Positively-charged surfaces also effectively adsorb organic matter (Davis, 1982; Gu et al., 1994), which is mainly anionic at typical groundwater pH values, and the adsorption of sufficient organic matter is presumed to be capable of reversing the surface charge of the ferric and aluminum oxyhydroxide coatings from positive to negative.

If organic matter adsorption can reverse the surface charge of aquifer grains, then organic matter should inhibit the removal of negatively-charged colloids and microbes. Most research, in the laboratory and in the field, shows this is true for colloids (Amirbahman and Olson, 1993; Kretzschmar et al., 1995; Ryan et al., 1999; Franchi and O'Melia, 2003), viruses (Fuhs et al., 1985; Powelson et al., 1990; Pieper et al., 1997; Zhuang and Jin, 2003a; Levy et al., 2007; Yuan et al., 2008), bacteria (Martin et al., 1991; Johnson and Logan, 1996; Dong et al., 2002; Hall et al., 2005), and oocysts (Dai and Hozalski, 2002, 2003). Inhibition of removal was mainly attributed to blocking of colloid and microbe attachment by organic matter adsorption on the porous medium, but some researchers attributed inhibition of removal to an increase in the negative surface charge of microbes caused by adsorption of organic matter to the microbe surfaces (Bixby and O'Brien, 1979; Dai and Hozalski, 2002, 2003). Adsorption of organic matter to microbes has been measured directly by a variety of methods (Bixby and O'Brien, 1979; Campbell et al., 1997; Fein et al., 1999; Parent and Velegol, 2004) or indirectly by an increase in negative zeta potential (Dai and Hozalski, 2002, 2003). In contrast, Liu et al. (2009) found that organic matter enhanced the removal of some *Cryptosporidium parvum* oocysts to organic matter-coated silica surfaces; they attributed this behavior to surface charge heterogeneity and the presence of positively-charged area on the organic matter-coated surface.

The goal of this research is to examine the influence of organic matter on the transport of *Cryptosporidium parvum*

oocysts through a ferric oxyhydroxide-coated quartz sand saturated porous medium and to ascertain the mechanism by which organic matter affects oocyst removal. To do this, we measured the effect of different concentrations of dissolved organic matter on the streaming potential of the porous medium and the zeta potential of the oocysts.

2. Materials and methods

2.1. *Cryptosporidium parvum* oocysts

Formalin-inactivated *Cryptosporidium parvum* oocysts were obtained from Sterling Parasitology Laboratory (SPL) at the University of Arizona at Tucson. We used formalin-inactivated oocysts in these experiments even though inactivation results in changes to oocyst deposition behavior (Kuznar and Elimelech, 2005) because we used formalin-inactivated oocysts in an intermediate-scale aquifer tank experiment in a laboratory that could not be secured for the use of active oocysts. The oocysts were shed from a calf infected with the Iowa isolate of *Cryptosporidium parvum*, purified at SPL by discontinuous sucrose and cesium chloride centrifugation gradients (Brush et al., 1998), re-suspended in a solution of 5% formaldehyde, 0.01% Tween 20, 0.85% NaCl, and three antibiotics (penicillin, 111 U mL⁻¹; streptomycin, 111 U mL⁻¹; gentamicin, 56 µg mL⁻¹), and stored at 4 °C. The oocysts were pelleted from the formalin solution by centrifugation (12,000 × g, 4 °C, 30 min) and re-suspended in the experimental solution (10⁻⁴ M NaCl, pH 5.6–5.8) at a concentration of about 10⁵ oocysts per milliliter for at least 2 weeks before the column transport experiments.

The oocysts were enumerated by epifluorescence microscopy. Samples containing oocysts were stained with DAPI (4,6-diamidino-2-phenylindole; 0.1 mg L⁻¹) for 15 min and filtered with vacuum assistance (0.34 bar) onto black polycarbonate membranes (1.0 µm pore diameter, GE Osmonics) overlaying nitrocellulose backing filters (0.45 µm pore diameter, Pall-Gelman). The membranes were mounted on glass slides and the oocysts were counted manually using an epifluorescence microscope (Nikon Optiphot-2, 788× magnification, 350 nm excitation, 470 nm emission). At least 20 fields of view with 10 oocysts per field, or a maximum of 50 fields with fewer than 10 oocysts per field, were counted.

The average diameter of the DAPI-stained oocysts was measured by flow cytometry and epifluorescence microscopy outfitted with image analysis (Image Technology Corporation, Deer Park, NY). Fluorescent polystyrene microspheres (0.2–6.0 µm; Polysciences) were used to calibrate both the flow cytometer (Biorad, HS Bryte) and the epifluorescence microscopy image analysis.

The buoyant density of the oocysts was determined by density gradient centrifugation in Percoll I solution (1.131 g mL⁻¹, Sigma Chemical Co.) following a procedure used by Harvey et al. (1997). The gradients were overlaid with 500 µL of an oocyst suspension at a concentration of about 1 × 10⁷ oocyst mL⁻¹ and density marker beads (synthetically derived from the polysaccharide dextran; Sigma Chemical Co.) in a range of 1.095–1.138 g mL⁻¹ prepared in 0.15 M NaCl. The gradient was developed by centrifugation

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