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Surfactant Modified Zeolite Can Protect Drinking Water Wells from Viruses and Bacteria

Septic tanks, sewage effluents, and landfills can release microbial pathogens into groundwater. This problem is amplified in the so-called colonias along the U.S.-Mexico border and other low-income areas around the world that have no public sewage systems. The result is often outbreaks of groundwater-associated disease for which enteric viruses and bacteria, spread via a fecal-oral route, are responsible. However, due to difficulties and limitations in detection and surveillance of disease outbreaks, the causative agents for more than 50% of the outbreaks are unknown, though the clinical features suggest a viral etiology for most of those cases [*U.S. Center for Disease Control and Prevention*, 1993]. Enteric pathogens such as *E. coli 0157:H7*, *Campylobacter*, Enteroviruses, Hepatitis A virus, and caliciviruses have been responsible for groundwater-related microbial infections in humans. Inexpensive solutions to this problem are urgently needed. The recent threat of bio-terrorism and concerns about the safety of drinking water supplies further add to that urgency.

A recent study shows that surfactant-modified zeolite (SMZ) can be used as a filter pack for drinking water wells to efficiently remove very high concentrations of viruses and bacteria from septic effluent. SMZ is economical—about $0.50/kg—and could significantly reduce the potential for viral contamination of drinking water wells. However, studies are needed to test the feasibility of using SMZ as a filter pack for long periods.

Viruses in Drinking Water

The primary factors controlling pathogen fate and transport in groundwater are inactivation, advection and dispersion, and attachment and detachment. For human enteric viruses, generally no growth occurs in the groundwater due to the lack of a host. The viruses will cease activity with time, depending on environmental conditions such as temperature, water chemistry, and dissolved oxygen. Advection and dispersion depend on the hydraulic properties of the aquifer. Attachment and detachment are complex processes in which a number of interactions between virions and porous medium particles are involved. Forces responsible for the attachment and detachment of viruses include electrostatic attraction and repulsion, van der Waals forces, and hydrophobic effects. Other factors contributing to the attachment of viruses onto porous media include Brownian movement and straining-the removal of particles due to pore size restrictions-and these depend on the size of virions and the medium particles.

Viruses possess a protein coat that results in a surface charge that varies with pH. The isoelectric point (pH_{iep}) of a virus particle is used to describe its net charge in the groundwater environment. The pH_{iep} is a specific pH value under which the virion has a net neutral charge. If the pH of water is greater than the pH_{iep} of a certain virion in water, the surface of the virus particle is negatively charged. The surface is positively charged if the pH of water is lower than the pH_{iep} of the virion. At typical groundwater pHs, most virions have a net negative surface charge. Due to their net positive charges, materials with high isoelectric points may be suited as geological barriers to virus migration. Ferric oxide-coated sand may serve as a suitable permeable barrier due to its high pH_{iep}. Clay minerals have large specific surface areas, but their generally negative structural charge limits their ability to adsorb viruses. If clay minerals are modified by certain organic surfactants, their surface charge becomes positive, and their hydrophobicity increases. Thus, their ability to adsorb many non-polar and anionic contaminants in water is amplified. However, the low permeability of clay minerals limits their suitability as permeable barriers for practical applications. Natural zeolites are a feasible alternative because, along with large specific surface areas and cation exchange capacities, they form stable aggregates that can be ground and sieved to any desired permeability.

Methodology and Approach

Iron oxide-coated sand (ICS), untreated zeolite (UZ), and surfactant-modified zeolite (SMZ) were tested for their suitability as permeable barriers to inhibit virus migration. Sand grains coated with ferric oxyhydroxides were prepared by adding anhydrous $FeCl_3$ solution to the sand and precipitating iron oxyhydroxides at high pH with the addition of NaOH. After several days of coating followed by rinsing through a 500-μm sieve to remove colloidal and fine particulates of iron oxide, the sand was dried at 95°C overnight. The iron coating increased the pH_{iep} of the sand—which consisted of a mix of quartz and feldspar minerals—from an average of 4 to about 8.5, reflecting the change to a positive surface charge.

In this study, a clinoptilolite-dominated zeolite was selected from the more than 40 naturally occurring zeolite minerals. About 70% clinoptilolite by weight, the zeolite had a specific surface area of 15 m^2/g, a particle size of 1.4–2.4 mm, and a bulk density of 0.9 g/cm^3. The zeolite surface area is about two orders of magnitude larger than that of the sand used in this study.

Due to isomorphic substitution by aluminum in the silicate crystal lattice, zeolites possess a net negative surface charge, which limits their capacity for adsorbing viruses. However, the surface charge can be reversed by treatment with long-chain cationic surfactants. For this study, the untreated zeolite (UZ) was modified by spraying a 30% aqueous solution (by weight) of hexadecyltrimethylammonium chloride (HDTMA-Cl) onto the zeolite, thoroughly mixing the slurry for 5 minutes, and drying the resultant surfactant-modified zeolite (SMZ) at 2500°C for 10 min, to yield SMZ with an HDTMA content of 140 mmol/kg. HDTMA is a bulk-production surfactant used in hair conditioners and mouthwash. The HDTMA quantitatively exchanges with charge-balancing inorganic cations on the external zeolite surface and forms a stable surfactant bilayer. Bilayer formation reverses the zeolite surface charge from negative to positive and creates a hydrophobic environment at the zeolite surface [*Bowman et al.*, 2000]. The anion exchange and organic partitioning properties of SMZ promote virus adsorption. The surface of SMZ is shown schematically in Figure 1.

The permeable barrier materials were tested both in the laboratory and in the field. The SMZ was found to be stable in ionic strength water larger than 5 millimoles (mM). Thus, the ionic strength of the water was controlled to be between 5mM and 15 mM for both the lab and field experiments. Bacteriophages were used instead of enteric viruses due to the biological hazards of the latter. Bacteriophages serve as good surrogates for investigating the behavior of human enteric viruses in the environment because of their similarities in dimensions, reported similarities in behavior to enteric viruses, and relatively low assay costs [*Sobsey et al.*, 1995; *Dowd et al.*, 1998; *Nasser and Oman*, 1999]. Particularly suited are MS2 and phix174. MS2 has a pH_{iep} of 3.9 and a diameter of 24 nm, while phix174 has a pH_{iep} of 6.6 and a diameter of 27 nm. The host bacteria for MS2 was *E. coli Famp*, while for phix174 it was *E. coli ATCC13706*, respectively. The titer of the phages in the water samples was tested within three days using the double-agar-layer method [*Adams*, 1959]. Bromide was used as a water tracer, and its concentrations were measured using capillary electrophoresis.

Laboratory Experiments

A model Plexiglas aquifer was used for the laboratory experiments. The model aquifer was filled with sieved play sand. The mineralogical composition of the sand was

70% quartz sand, 10% feldspar, and 20% volcanic rock fragments with an elemental composition similar to feldspar. The porosity of the sediment was 0.48, and the hydraulic conductivity was 1.3×10^{-3} m/s. Ten piezometers for injection and sampling were aligned with equal spacing along the main flow line through the model aquifer. The experimental runs were completed under room temperature, 20–25°C, which match groundwater temperatures in shallow aquifers near the Mexico-U.S. border. The hydraulic gradient was controlled by manipulating the water levels in the inlet and outlet reservoirs. Before each experimental run, the model aquifer was flushed with 5 pore volumes of de-ionized water and background samples for pH, bromide and phages were collected from the injection well and sampling ports. MS2 and phix174 (10^6pfu/ml) and bromide (1000 mg/l, as NaBr) were injected together into the aquifer. Water samples were collected from the sampling ports based on predictions of a finite-difference computer model that was used to simulate bromide migration rates. The samples were stored at 4°C until analysis. The model aquifer results included one control run (without a barrier), one run with UZ, one with ICS, and two runs with SMZ as permeable barriers.

Separate laboratory studies were conducted to distinguish adsorption versus inactivation of MS2 coliphage by SMZ. For the adsorption study, defined numbers of coliphages were added to aqueous suspensions of SMZ in separate tubes. The tubes were periodically removed and centrifuged to separate the adsorbed phages from those in suspension, and the numbers of suspended phages were enumerated. For the inactivation studies, SMZ was equilibrated with water in the absence of coliphage. The equilibrated water was separated from the SMZ and inoculated with coliphage in separate tubes. Coliphage inactivation over time was determined for these SMZ-free samples. The sorption and inactivation studies both were conducted by mixing at room temperature for 120 min using a rotary laboratory shaker.

Field Experiments

The field experiments were conducted at a site where sewage effluent was discharged from a septic system into a constructed submerged wetland. The constructed wetland had dimensions of 7.62 m x 3.05 m and was filled with pea gravel with a grain size of 0.95 cm. The gravel depth ranged from 30.5 cm to 39.6 cm from the influent to the effluent side. Twenty-five milliliters of MS2 at a concentration of about 10^{10} pfu/ml and 2 ml of *E. coli* at a concentration of 10^7 cfu/ml were diluted in 3.785 L of deionized water enriched with 300 mg/L NaCl solution to prevent osmotic shock. A 1 ml water sample from each container was collected to measure concentrations before injection. Forty grams of NaBr were diluted in 3.785 L in a separate container of water and used as a conservative tracer. MS2, *E. coli*, and bromide were then injected together into the groundwater at the influent side of the wetland. A well at the downgradient side of the wetland was used to monitor effluent concentrations. Effluent concentrations were also measured at the outflow of the constructed wetland.

The first field test was conducted without any barrier material. The effluent well was used solely as a monitoring well. In the tests using ICS and SMZ as barrier material, the effluent well was pumped at a rate of 12 ml/s to mimic a water supply well used for domestic use. With this rate of extraction, approximately two-thirds of the discharged water from the wetland went through the effluent well and one-third of the water went through the constructed outflow. The permeable barrier materials were used as filter packs for the effluent well. The filter pack had a thickness of about 10 cm, and the residence time of the water in the filter pack was about 2 min.

This design allowed evaluation of the efficiencies of ICS and SMZ in removing viral and bacterial concentrations from the septic groundwater by comparing ICS and SMZ results to the control run without any barrier and comparing the water analysis results from the effluent well to that of the outflow of the constructed wetland.

Results

The data from the model aquifer experiments are summarized in Figure 2. Untreated zeolite failed to remove the more negatively charged MS2 to a significant extent. Fe-oxide coated sand did remove phix174 and MS2 to a large extent. SMZ completely removed both viruses as well as bromide.

In the field tests, Fe-oxide coated sand did not perform well as a filter pack material. Compared to Fe-oxide coated sand, SMZ removed about 100 times more viruses (Figure 3a). The high efficiency of SMZ can also be shown by comparing the water withdrawn from the effluent well with water from the outflow. SMZ removed at least 99% of the viruses in the water (Figure 3b). In addition, the SMZ removed 100% of E. coli present in the water (Figure 3c), while the ICS did not show any effect on *E. coli* concentrations.

The sorption and inactivation tests indicate that virus titers declined significantly because of the combined effects of sorption and inactivation by SMZ (Figure 3d). While most of the viruses were removed by sorption, the inactivation of viruses in the presence of SMZ-derived HDTMA within 120 minutes is significant considering that under the field conditions, the viruses could have been exposed to dissolved surfactant for even longer periods of time. Natural inactivation, on the other hand, was shown to be insignificant for the time scale of these experiments (Figure 3d).

Based on both laboratory and field results, SMZ performed very well in removing viruses from the groundwater. The only concern is the non-selectivity of SMZ; it removes both viruses and negatively charged ions such as bromide, which may limit the applicability of SMZ in high-salinity waters and in long-term applications. However, we expect large, multi-valent virus anions to be selectively retained by the SMZ [*Li and Bowman*, 1997]. ICS removed viruses very efficiently in the lab experiments and on a selective basis, but it failed to remove a significant amount of viruses in the field tests.

Untreated zeolite is not a suitable material for removing viruses because of its net negative surface charge. ICS performed surprisingly poorly in the field tests. Virus and *E. coli* concentrations were even higher than during the control run, most likely due to the different hydraulic conditions when the effluent well was used as pumping well. The reason for the poor performance of ICS in the field test can mostly be attributed to the attachment of viruses and bacteria to organic matter in the septic effluent. Viruses and bacteria attached to organic matter would not sorb onto hydrophilic Fe-oxide coated sand, but could be removed by the hydrophobic surfactant coating of the zeolite. Also, other particles or chemical species present in the wetland may have interacted with the iron coating.

Based on the sorption and inactivation results, most of the virus removal by SMZ can be attributed to sorption. This observation is also consistent with model aquifer tests conducted without permeable barriers, where both irreversible and reversible sorption occurred at significant levels [*Schulze-Makuch et al.*, 2002]. The presence of reversible sorption in the field tests can be inferred from the delayed breakthroughs for both ICS and SMZ compared to the bromide migration rate. The retardation factors of MS2 in Fe-oxide coated sand and SMZ in the field tests were 1.3 and 1.7, respectively. Aside from irreversible and reversible sorption, virus inactivation by surfactant in aqueous solution also appears to be a significant process. A minor amount of the surfactant coating detached and dissolved into the water, thus probably removing a significant percentage of the viruses in the field test. Natural inactivation, on the other hand, appears to be negligible on the time scales considered here. However, eventually the attached viruses are deactivated, probably on a time scale of tens of days [*Keswick et al.*, 1982].

The most intriguing result is the complete removal of E. coli by SMZ during the field tests. The surfactant used to prepare SMZ, HDTMA, is bactericidal in aqueous solution even at low concentrations. However, if sorbed, E. coli may still stay alive for an unknown period of time, because the toxicity of HDTMA to the organisms is greatly reduced or eliminated if the surfactant is bound to zeolite [*Fuierer et al.*, 2001].

Future Research

Further studies are needed to investigate whether viable viruses and bacteria are released from the SMZ over time and to determine the lifetime of an SMZ filter pack. Also, since surfactant slowly washes off from SMZ, the potential adverse health effects of low levels of HDTMA in the treated water need to be determined. There is currently no drinking water standard for HDTMA and it is approved for use in personal products such as mouthwash and hair conditioners.

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Fig. 1. This schematic shows anion exchange and organic partitioning on surfactant modified zeolite (SMZ).

Fig. 2. Effect of various permeable barriers on virus and bromide concentrations in the model aquifer is shown. W1 is the monitoring well upgradient of the barrier, and W2 the monitoring well downgradient of the barrier. Bromide concentrations are given on the primary y-axis; virus concentrations are given on the secondary y-axis. Due to some technical problems, phix174 concentrations are not available for the zeolite and SMZ-1 run.

Fig. 3. Results of field and deactivation tests are shown (pfu = plaque forming units; cfu = cell forming units; dashed lines = interpreted breakthroughs). The high virus removal efficiency of the SMZ filter pack can be shown by comparing it to the Fe-coated sand filter pack (panel a) and by comparing virus concentrations from the effluent well with water from the constructed outflow (panel b). The SMZ filter pack also removed 100 % of E. coli in the water (panel c). Sorption and inactivation tests revealed that the virus removal can be attributed mostly to sorption and to a lesser degree to inactivation due to surfactant in water (panel d).