Groundwater Research Report
WR12R003

TRANSPORT OF MANURE-DERIVED ESCHERICHIA COLI WITHIN NATURALLY-FRACTURED DOLOMITE

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Title: Transport of Manure-derived *Escherichia coli* within Naturally-Fractured Dolomite

Project ID: WR12R003

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Background/Need: The shallow fractured dolomite aquifer represents a major source of domestic water supply in NE Wisconsin. High percentages of domestic wells, however, were found to be contaminated by bacteria and manure was identified as the most likely source. A thorough understanding of the transport behavior of manure-derived bacteria within this aquifer is critical to the assessment of groundwater microbial contamination risks and design and implementation of appropriate mitigation options. However, there is a lack of information on the mechanisms and processes that govern the movement of bacteria within fractured dolomite materials under various water chemistry and flow conditions. This proposed research aims at filling this knowledge gap. Findings from this research will improve our capability of modeling the transport of manure-derived bacteria within fractured dolomite aquifers, and can be used to design new regulation guidelines (e.g., the location and timing of manure application) and to identify wells that are most susceptible to microbial contamination due to manure application.

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1. Introduction

As one of the largest producers of milk and milk products in the United States, Wisconsin is the home to ~15,000 dairy farms and ~1.2 million cows (USDA 2010a) . Each year the dairy cows in Wisconsin produce ~4 million tons (dry weight) of manure (USDA 2010b). Additionally, ~ 2 million tons (dry weight) of manure is produced by Wisconsin's cattle, swine and poultry farms (USDA 2010b). Manure produced on the animal farms is usually applied to the crop fields as a source of fertilizer. The spread of manure is often performed either daily or following temporary storage in structures that are often not lined (Turnquist et al. 2006, Jackson-Smith et al. 2000). Thanks to the large quantities (e.g., *E. coli*: ~10^8 cell/g of wet manure) of bacteria such as *E. coli* (Duriez and Topp 2007, Reddy et al. 1981, Sinton et al. 2007, Walczak and Xu 2011) that manure contains, leakage from manure storage structures and downward infiltration of water through manure-laden soil could lead to groundwater microbial contamination.

In Northeastern (NE) Wisconsin (e.g., Brown county, Calumet county, Door county, Kewaunee county and Manitowoc county), the shallow fractured dolomite aquifer, which represents a primary source of domestic water supply, is particularly susceptible to microbial contamination as a result of manure application because in many areas the soil layer on top of the aquifer is fairly thin (Erb and Stieglitz 2007). A survey performed during 2002-2005 in Calumet county showed that 35% of private well samples were positive for coliform bacteria (Erb and Stieglitz 2007). In general, bacteria leached out from manure were identified as the most likely source of the microbial contamination. The microbial contamination in the dolomite aquifer represents a major threat to public health. A thorough understanding of the transport behavior of manure-derived bacteria in fractured dolomite aquifer system is critical to the delineation of highly susceptible areas, the assessment of groundwater contamination and public health risks, as well as the design, evaluation and implementation of mitigation options.

To our knowledge, little is known about the factors and processes that govern the transport of manure-derived bacteria (even bacteria in general) in the fractured dolomite aquifer in NE Wisconsin. The primary goal of this research is to fill this knowledge gap through experimentally investigating the transport of manure-derived *E. coli*, the most commonly used indicator microorganism for fecal contamination, within the fractured dolomite system. Our approach consists of a series of bench-scale transport experiments using naturally-fractured blocks of dolomite.

Findings from this proposed research allowed us to gain an improved understanding of the mechanisms that control the retention and release of *E. coli* within natural dolomite fractures and will provide useful kinetics parameters for the future development, refinement and validation of predictive mathematical models. Combined with field observation data (e.g., water chemistry), the results obtained from this research can also provide a basis for evaluating the public health risks associated with the application of manure as a fertilizer in agricultural fields (e.g., to identify the private wells that are most susceptible to microbial contamination). Additionally, the findings can lead to improved manure management practices, such as the timing of manure application and the selection of agricultural fields for manure application, which can potentially reduce the risks of groundwater contamination by manure-derived bacteria.

Fractured carbonate aquifers represent important sources of drinking water in areas beyond the state of Wisconsin (Muldoon and Bradbury 2005, McKay 2011) and bacterial

contamination of fractured rock aquifers by livestock manure has been documented in many other places (e.g.,Masciopinto et al. 2008, Levison and Novakowski 2009). The findings from this project will thus have broad applications by advancing our understanding of the spread of bacteria in saturated fractured geological formations.

2. Research Goals

It is well known that groundwater movement and contaminant transport in the dolomite aquifer in Eastern Wisconsin occurs predominately through the interconnected fracture networks (Muldoon and Bradbury 2009) and such advective transport through the fracture systems can be very rapid. This main goal of this research is to quantify the transport of *Escherichia coli*, a representative indicator bacterium for fecal contamination, through natural fractures in dolomite at the laboratory scale under a range of water chemistry and flow conditions. More specifically, this study will address the following objectives:

(1) To determine the effect of pore water chemistry and flow velocity on the mobility of manure-derived *E. coli* in fractured dolomite.

(2) To quantify the release of previously retained *E. coli* cells due to chemical (i.e., change in pore water chemistry) or flow perturbations in fractured dolomite.

3. Materials and Methods:

3.1 Preparation of Fractured Dolomite Samples

Figure 1. Illustrative photo of fractured dolomite. The horizontal fractures are clear from this photo. The thickness of each layer is ~20-30 cm. The rectangle represents a potential fractured dolomite sample that can be used for the bacterial transport experiment.

The fractured dolomite samples used for the transport experiments were obtained from a

Halquist quarry (Figure 1) which is located near Sussex, WI. With the help of the operator of the quarry, fractured dolomite samples (~1ft in cube) were obtained from the quarry with minimal disturbance (Figure 2).

Figure 2. Photo of fractured dolomite samples obtained from the quarry. The horizontal fractures are clear from this photo. Smaller horizontal fractures that are not readily observable in the photos are also present.

Upon the delivery of a dolomite block to our lab at UWM, custom-made Acrylic end caps (with viton O-rings and rubber liners) were fixed to the upstream (e.g., right) and downstream (e.g., left) ends of the horizontally oriented fracture. Small chambers were carved from the end caps to distribute the flow. The other two sides of the fracture plane were sealed using Acrylic plates (with rubber liners) to create no-flow boundaries (Figures 3 and 4) (Dickson and Thomson 2003). Similar designs have been successfully used to study the transport of contaminants (e.g., trichloroethylene) within fractured rocks (Dickson and Thomson 2003). Using a three-way valve, a pair of syringe pumps (New Era Syringe Pump Systems, Farmingdale, NY) were used to alternately inject fluid (e.g., cell suspension or background solution) into the fracture plane, while fluid that exits the system at the downstream end could be collected using fraction collectors (Spectrum Spectra/Chrom CF-1) or directed to flow-through spectrophotometer cells for the quantification of chemicals and/or bacterial cells. The use of two syringe pumps will allow for 1) the injection of large volumes (if needed) of liquid into the fractured dolomite sample (as one pumps is close to become empty, the other pump will be switched on) and 2) for the case of *E. coli* suspension, the minimization of cell settling (each pump will be used for short period of time while the other pump will be filled with fresh and thoroughly mixed cell suspension).

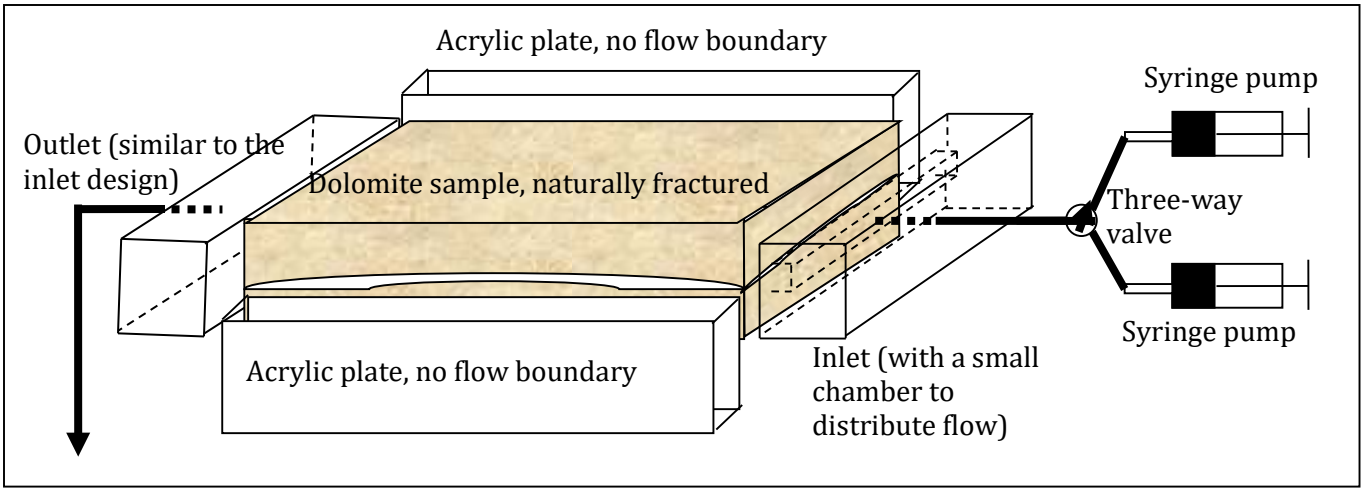
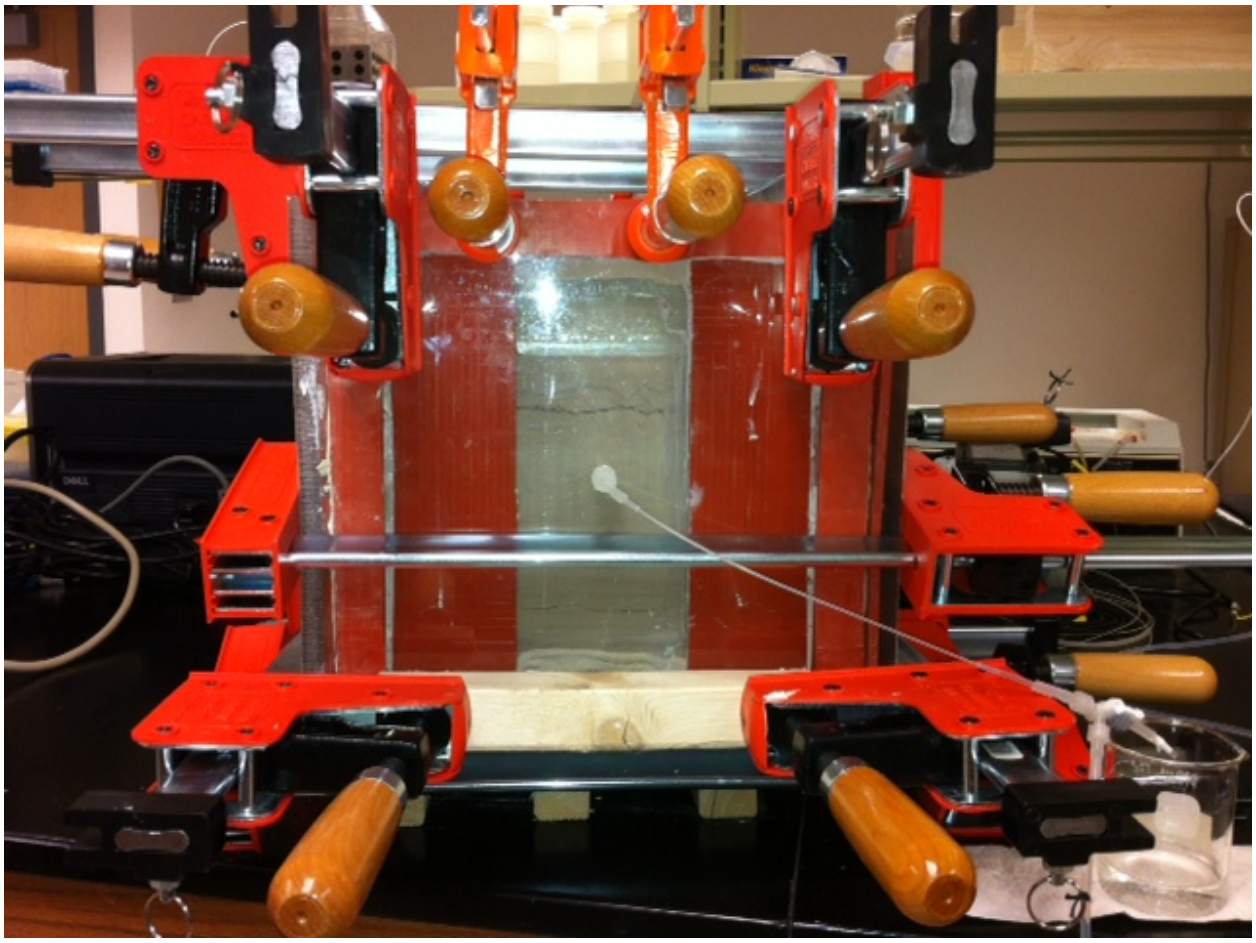


Figure 3. Schematic diagram of the setup for the transport experiments using dolomite samples with natural fractures.



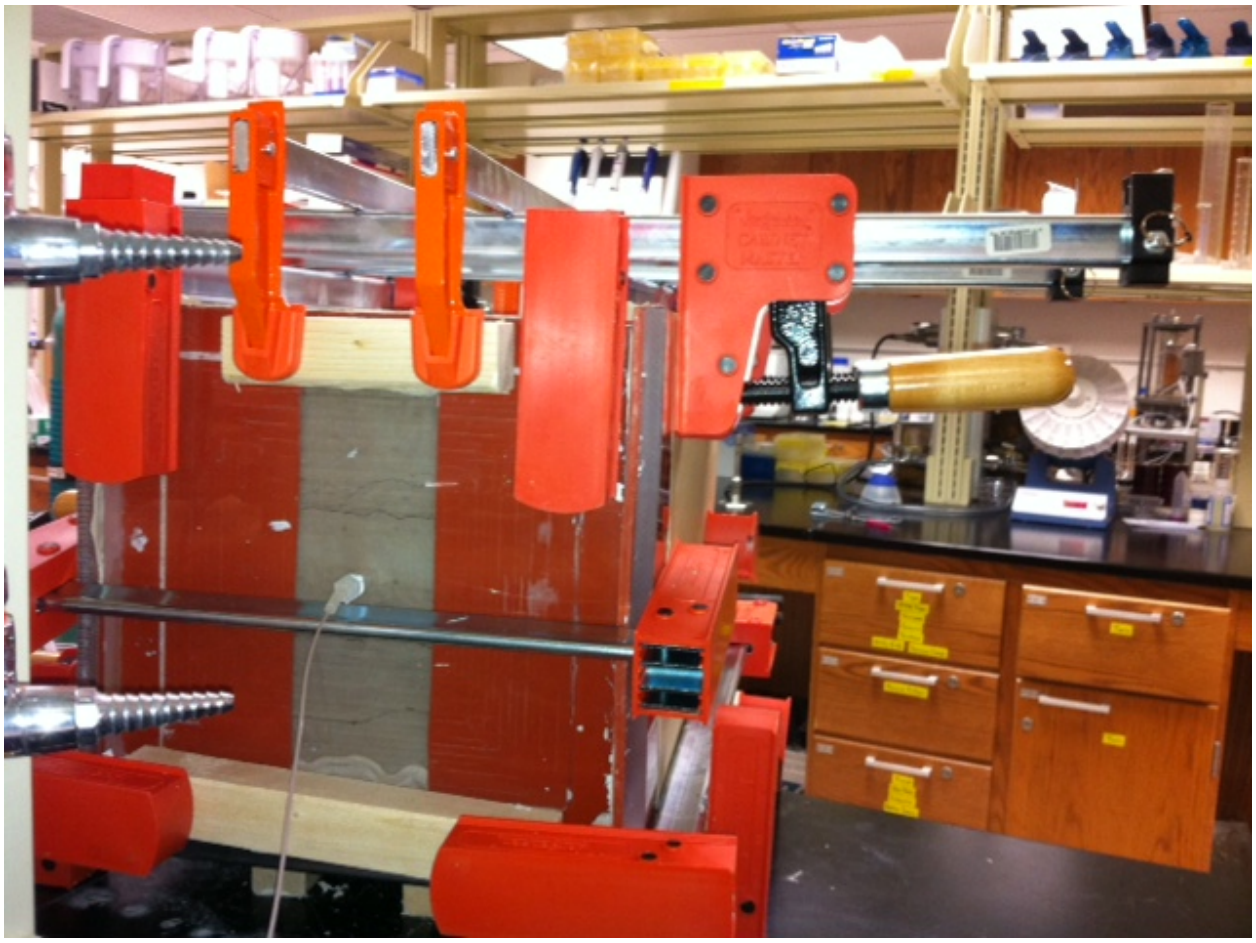


Figure 4. Front (top) and back (bottom) views of the sealed fractured dolomite sample based on the sketches shown in Figure 3. The injection port are located at the center of the front and back panels. The horizontal fractures are observable through the acrylic side plates.

3.2 Tracer Tests

Tracer tests were firstly performed using 0.25 mM $NaNO_3$ (9.75 mM of NaCl as background). The tracer solution was injected into the dolomite sample and effluent were directed to quartz flow-through cells housed in a Shimadzu UV/Vis1800 spectrophotometer. The nitrate concentrations in the effluent were determined using a wavelength of 220nm (Porubcan and Xu 2011). The injection lasted 105 minutes. Following the injection step, 10 mM NaCl solution was used to flush the dolomite block. During the rinsing stage, the concentrations of nitrate was periodically checked to assure that the removal of the tracer is complete. According to Pulin Mondal (University of Toronto, personal communication), the rinsing step should have minimal effects on the properties of fractured dolomite (Mondal and Sleep 2011).

A fundamental difference between transport experiments performed using dolomite blocks and other natural porous media such as soil and sediment is that each dolomite block is unique in fractures size, distribution and structures and it is nearly impossible to find two or more identical blocks as replicates. The dolomite block therefore was reused in our experiments. The advantage of sample reuse was that the results obtained using one sample will be directly comparable. The challenge was to make sure that the sample can be thoroughly cleaned and

renewed so experiments performed at a later time were not affected by proceeding experiments. Mondal and Sleep (2011) used a single fractured dolomite block and found that the results remained reproducible for more than 10 reuses (Pulin Mondal, University of Toronto, personal communication). In this research, tracer tests were performed upon the completion of all bacterial transport experiments, and as the results showed, there was minimal change in the tracer test results, suggesting that the hydrological conditions of the sealed dolomite sample remained stable during the course of the experiments.

3.3 **Transport of *E. coli* through the Fractured Dolomite**

The *E. coli* strain used in this research was previously isolated from dairy manure (Walczak and Xu 2011, Walczak et al. 2011). An *E. coli* strain that previously displayed high mobility within sands was selected for this research (Walczak et al. 2011). The *E. coli* isolate stored in 20% glycerol under -80^{0}C were streaked onto Luria-Bertani (LB) agar plates. After overnight incubation at 37^{0}C, cells from the freshly formed colonies will be transferred to culture tubes containing 15 ml LB broth. The culture tubes were incubated at 37^{0}C (90 rpm shaking). After 18 hours of incubation, the bacterial cells were harvested using centrifuge (5000*g*, 10 minutes, 4^{0}C) and combined. To remove the growth medium, the combined bacterial pellet were rinsed 4 times with the appropriate electrolyte solution (Walczak et al. 2011). The concentration of cells were then adjusted to ~10^7 cells/ml and the suspension was ready for the transport study.

The *E. coli* transport experiment was initiated by introducing *E. coli* cell suspension into the upstream end of the dolomite plane using syringe pumps. Effluent samples were collected using a fraction collector. The effluents of the columns were connected to flow-through quartz cuvettes (NSG Precision) and the cell concentration was determined at a wavelength of 220nm at 30-s intervals using a spectrophotometer (Shimadzu UV-1700). After 60 minutes of injection (~3.5 pore volumes), the columns were flushed with background electrolyte solution until the absorbance of effluent returned to the background values.

To investigate the effects of ionic strength on the transport of *E. coli* within the fractured dolomite, the following water chemistry conditions were used: 3, 10 and 30 mM NaCl. To examine the effects of Ca^{2+} ions on *E. coli* mobility, 0.5, 2.5 and 5 mM $CaCl_2$ were used while the total ionic strength was maintained at 30 mM. the flow rate was maintained at 5.08 mL/min, which is equivalent to ~22 m/day.

4. Results and Discussion

4.1 Tracer Tests

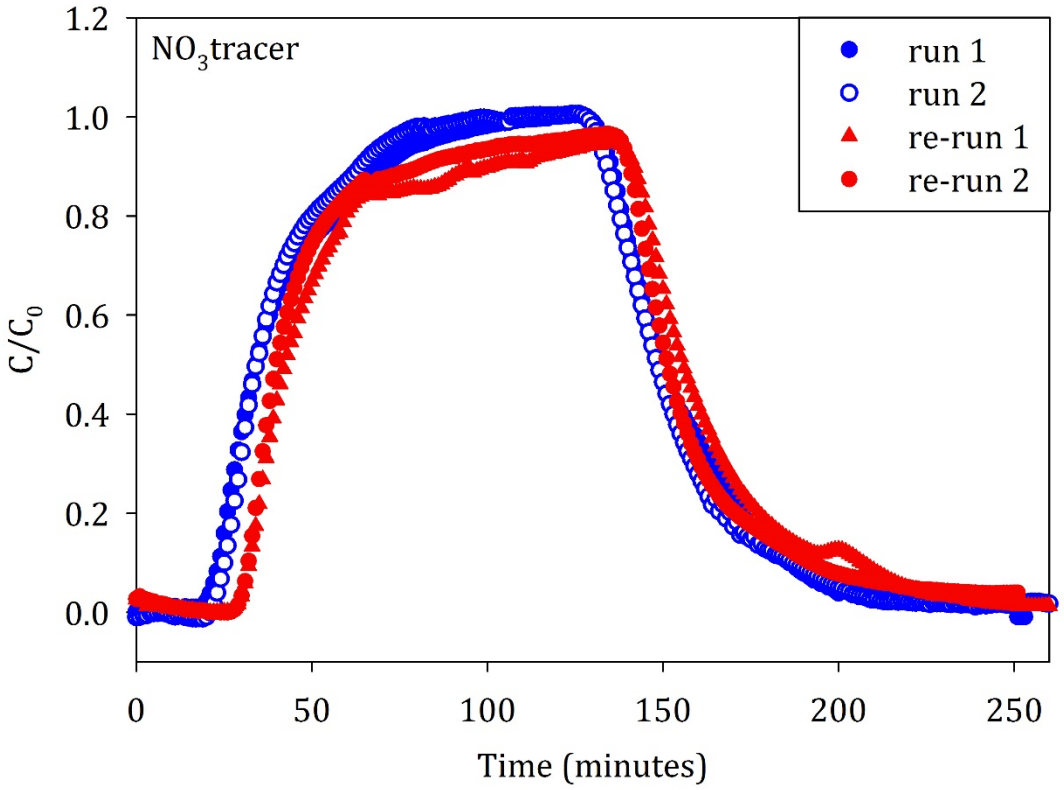


Figure 5. Results of tracer tests at the beginning (run 1 and 2) of the transport experiments (i.e., before any *E. coli* transport experiments) and upon the completion of the *E. coli* transport experiments (re-run 1 and 2).

Figure 5 shows the results of the tracer tests. There is minimal change in the breakthrough curves before and after the *E. coli* transport experiments, suggesting that the fractures within the dolomite block experienced negligible changes during the course of the experiments. The integration of the breakthrough curves indicated that ~100% of the injected tracer (i.e., nitrate) was recovered in the effluent, confirming the conservative behavior of the tracer within the dolomite block. During the injection stage, the concentration of the tracer rose rapidly, suggesting the relative small role played by dispersion. The tailing in the breakthrough curve indicated that the diffusion of the tracer into smaller fractures and pores during the injection phase and when the fractured dolomite was flushed using tracer free background electrolyte solutions, the previously trapped nitrate ions slowly diffused out from the low mobility zone.

4.2 *E. coli* Transport

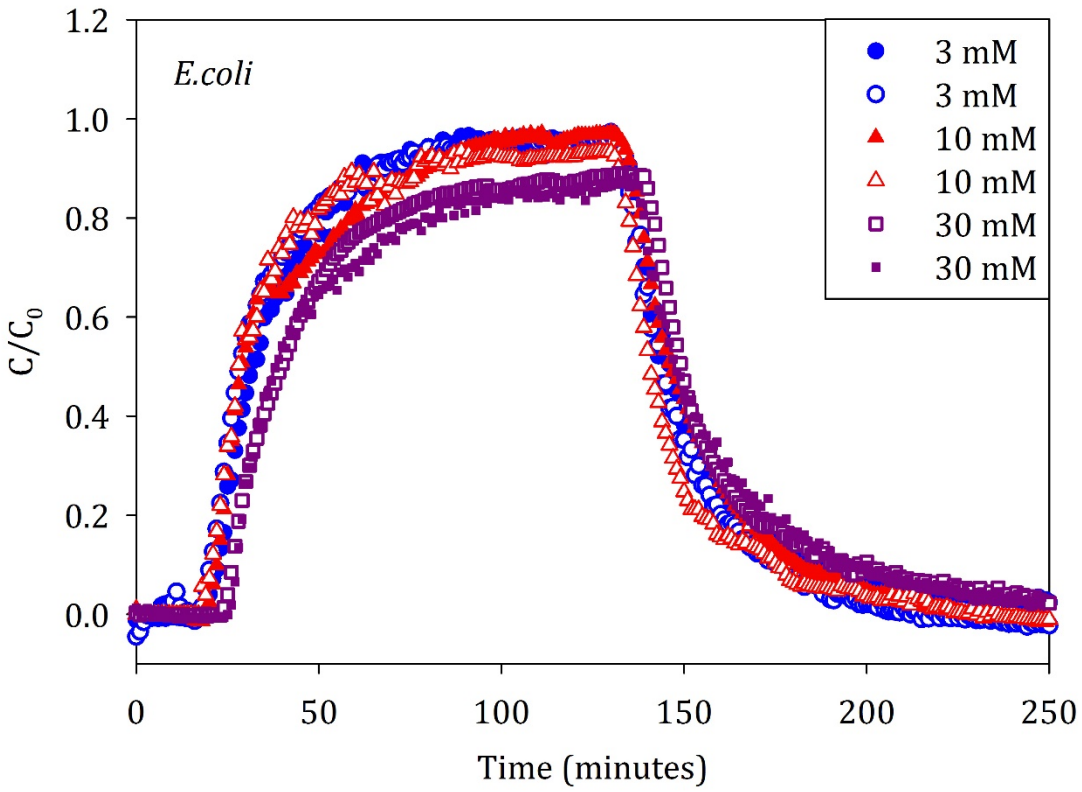


Figure 6. Breakthrough curves of *E. coli* transport through the fractured dolomite under various ionic strength conditions.

The breakthrough concentrations of manure-derived E. coli when traveling through the fractured dolomite under different ionic strength conditions (3-30 mM NaCl, or TDS 175.32-1753.2 mg/L) are shown in Figure 6. The *E. coli* breakthrough curves shared significant similarity to those of the conservative tracer, especially under low ionic strength conditions (3 and 10 mM NaCl). Therefore, there was negligible immobilization of the suspended *E. coli* cell as they travel through the fractures.

There was a significant decrease in *E. coli* breakthrough concentrations when the ionic strength increased from 10 mM to 30 mM (TDS from 584.4 mg/L to 1753.2 mg/L). The integration of the breakthrough curves showed that ~10% of the injected *E. coli* cells were immobilized. The immobilization of the *E. coli* cells with dolomite thus was significantly lower than their immobilization within natural quartz sands (Walczak et al. 2011).

The immobilization of bacterial cells within natural porous media including fractured rocks usually is achieved through a two-step process (Yao et al. 1971, Rajagopalan and Tien 1976, Tufenkji and Elimelech 2004): the cell strikes the surface of the solid phase, and the successful attachment of the cell to the surface of the solid matrix. The first step is controlled by advection (when the cell travels with flow and strike the surface of the solid matrix), diffusion

and sedimentation (if the cell is denser than the liquid). Under the flow conditions employed by this research, the flow within the fractures should be dominated by laminar flow. This would limit the chances that a cell could strike the surface of the solid matrix and thus could lower the cell immobilization efficiency. Additionally, when a cell strikes the surface of the solid matrix, the immobilization is dependent on the successful attachment of the cell to the surface, which in turn is controlled by the energy interactions between the cell and the surface (Johanson et al. 2012). My group is currently pursuing this energy interaction and cell deposition on the surface of dolomite using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) technology. The extended Derjaguin-Landau-Verweu-Overbeek (XDLVO) theory will be applied to gain a quantitative understanding of the energy interactions between the *E. coli* cells and the dolomite surface.

Calcium ions are ubiquitous in the fractured dolomite system due to the dissolution of dolomite in water. To examine the effects of Ca^{2+} on the transport of E. coli cells within fractured dolomite, a series of experiments were performed: the total ionic strength was maintained at 30 mM, while the concentration of Ca^{2+} increased from 0.5 mM to 5 mM. The results are shown in Figure 7.

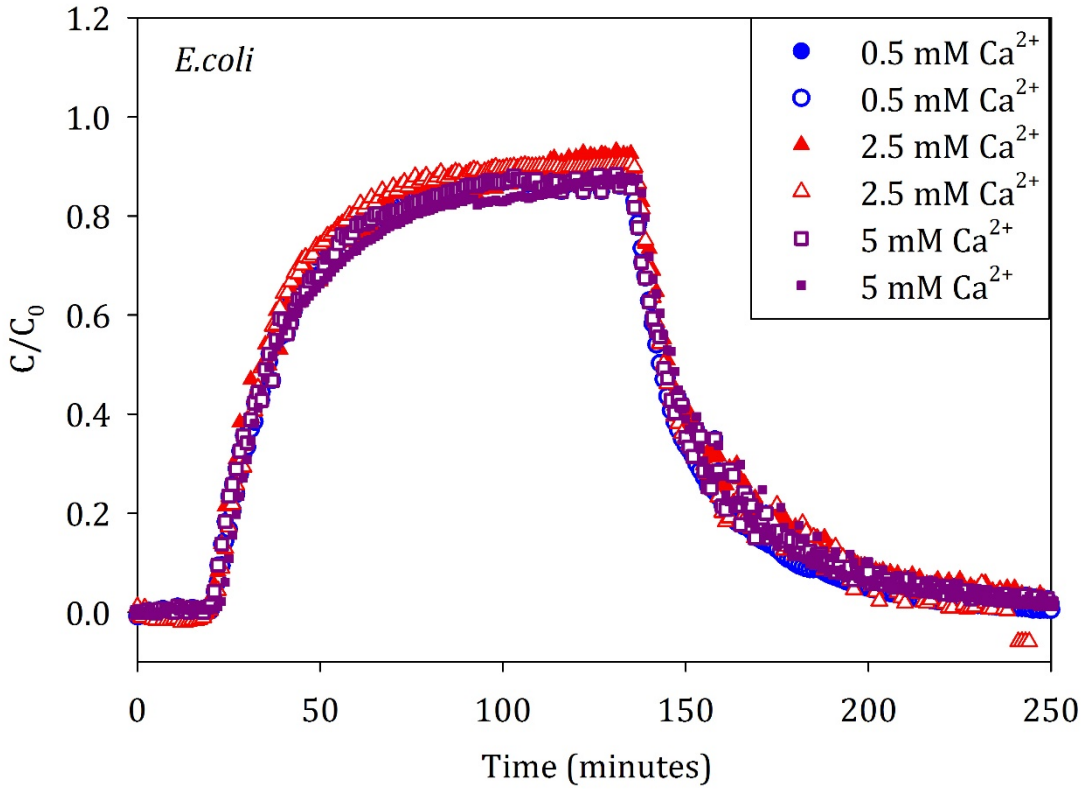


Figure 7. Effects of Ca^{2+} on the transport of *E. coli* through fractured dolomite. The total ionic strength was maintained at 30 mM using NaCl.

It was previously shown that Ca^{2+} can significantly enhance the immobilization of bacterial cells within natural sands because Ca^{2+} can alter cell surface properties (e.g., shielding charges that are responsible for repulsive interactions between the cell and the surface of the solid) (Walker and Kim 2009). Interesting, in this research, we observed that Ca^{2+} had negligible effects on *E. coli* immobilization within fractured dolomite. We attributed this insensitivity to the presence of Ca^{2+} ions as a result of dolomite dissolution even without the addition of Ca^{2+}.

5. Conclusion

In conclusion, our experimental results showed that manure-derived *E. coli* displayed high mobility (and low immobilization) within naturally fractured dolomite block under even high ionic strength conditions. For areas where the dolomite aquifer is impaired by bacteria leaching as a result of manure application, the bacterial cells can spread widely and quickly within the dolomite aquifer. Remediation effort, therefore, should be focused on reducing the possibility of bacterial leaching, or reducing the density of bacterial cells in the manure (e.g., through pre-treatment of manure such as fermentation).

6. References

USDA (2010a) National Agricultural Statistics Service: Livestock and Animals Database: : http://www.nass.usda.gov/QuickStats.

USDA (2010b) Confined Animal and Manure Nutrient Data System: http://www.ers.usda.gov/Data/manure/default.asp?ERSTab=0.

Turnquist, A., Foltz, J. and Roth, C. (2006) Manure management on Wisconsin farms, p. 23, Program on Agricultural Technology Studies, College of Agricultural and Life Sciences, University of Wisconsin-Madison.

Jackson-Smith, D., Moon, S., Ostrom, M. and Barham, B. (2000) Farming in Wisconsin at the end of the century: results of the 1999 Wisconsin farm poll, p. 18, Program on Agricultural Technology Studies, University of Wisconsin, Madison.

Duriez, P. and Topp, E. (2007) Temporal dynamics and impact of manure storage on antibiotic resistance patterns and population structure of Escherichia coli isolates from a commercial swine farm. Applied and Environmental Microbiology 73(17), 5486-5493.

Reddy, K.R., Khaleel, R. and Overcash, M.R. (1981) Behavior and Transport of Microbial Pathogens and Indicator Organisms in Soils Treated with Organic Wastes Journal of Environmental Quality 10(3), 255-266.

Sinton, L.W., Braithwaite, R.R., Hall, C.H. and Mackenzie, M.L. (2007) Survival of indicator and pathogenic bacteria in bovine feces on pasture. Applied and Environmental Microbiology 73(24), 7917-7925.

Walczak, J.J. and Xu, S.P. (2011) Manure as a Source of Antibiotic-Resistant Escherichia coli and Enterococci: a Case Study of a Wisconsin, USA Family Dairy Farm. Water Air and Soil Pollution 219(1-4), 579-589.

Erb, K. and Stieglitz, R. (2007) Final Report of the Northeast Wisconsin Karst Task Force.

Muldoon, M. and Bradbury, K.R. (2005) Site characterization in densely fractured dolomite: Comparison of methods. Ground Water 43(6), 863-876.

McKay, L.D. (2011) Foreword: Pathogens and Fecal Indicators in Groundwater. Ground Water 49(1), 1-3.

Masciopinto, C., La Mantia, R. and Chrysikopoulos, C.V. (2008) Fate and transport of pathogens in a fractured aquifer in the Salento area, Italy. Water Resources Research 44(1).

Levison, J. and Novakowski, K. (2009) The impact of cattle pasturing on groundwater quality in

bedrock aquifers having minimal overburden. Hydrogeology Journal 17(3), 559-569.
Muldoon, M.A. and Bradbury, K.R. (2009) Assessing seasonal variations in recharge and water quality in the Silurian aquifer in areas with thicker soil cover.
Dickson, S.E. and Thomson, N.R. (2003) Dissolution of entrapped DNAPLs in variable aperture fractures: Experimental data and empirical model. Environmental Science & Technology 37(18), 4128-4137.
Porubcan, A.A. and Xu, S.P. (2011) Colloid straining within saturated heterogeneous porous media. Water Research 45(4), 1796-1806.
Mondal, P.K. and Sleep, B. (2011) Effects of water ionic strength on virus and virus-sized particle transport in a variable-aperture dolomite rock fracture, p. 667, Minneapolis, MN.
Walczak, J.J., Bardy, S.L., Feriancikova, L. and Xu, S.P. (2011) Influence of tetracycline resistance on the transport of manure-derived Escherichia coil in saturated porous media. Water Research 45(4), 1681-1690.
Johanson, J.J., Feriancikova, L. and Xu, S.P. (2012) Influence of Enterococcal Surface Protein (esp) on the Transport of Enterococcus faecium within Saturated Quartz Sands. Environmental Science & Technology 46(3), 1511-1518.
Walker, S.L. and Kim, H.N. (2009) Escherichia coli transport in porous media: Influence of cell strain, solution chemistry, and temperature. Colloids and Surfaces B-Biointerfaces 71(1), 160-167.