Influence of Bacterial Pathogen Condition on Cell Transport in Groundwater Environments

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Due to California's continuing population growth driving demand for an increased municipal water supply and political pressures for decreased reliance on Colorado River water, there is a vital need to ensure groundwater protection and quality. In the area of the Santa Ana Regional Water Quality Control Board, a region of approximately 2,800 square miles and a population approaching 6 million people, reclaimed wastewater is increasingly utilized for groundwater recharge. Additionally, non-point source pathogen pollution is a mounting problem due to sources such as dense dairy farming and urban runoff. For water quality professionals in the Santa Ana Region and beyond, the capacity to determine the transport and source of bacterial pathogens is essential to safeguard drinking water supplies. Therefore, the ability to predict the fate of human pathogens in the environment is critical, and a mechanistic understanding of bacterial transport in the subsurface environment is imperative for assessing the environmental impact of groundwater contamination from sources including urban runoff, septic tank/leach field systems, and animal manure from agricultural operations. Additionally, such information is vital for effective design of water quality technologies including riverbank filtration, wastewater reclamation, and recharge into aquifers.

A systematic and extensive examination of the *physiological and environmental factors* controlling bacterial adhesion and transport in subsurface environments is actively being pursued. Currently we are investigating the role that extracellular polymeric substances (EPS) exuded by bacterial pathogens plays in controlling the adhesive nature of the cells – expressly analyzing the composition of the EPS and adhesion trends in a flowing environment as a coupled phenomenon. This novel approach of investigating the dynamic surface chemistry of bacterial cells and the extent of adhesion will provide a more complete understanding of bacterial pathogen transport mechanisms.

The project is conducted in two core areas. The first is the development of methodologies to extract and analyze the composition of the EPS. These methods were developed in the past; however, the various methods have limitations of efficiency, detection, and reproducibility that need to be worked out and optimized for analyzing EPS of groundwater-borne pathogens. Work to date has involved testing extraction methods involving ethanol, lyophilization (or freeze-drying), and sonication. The compositional analysis involves traditional spectroscopic quantification techniques; however new methods using HPLC and gas chromatography are currently being explored. The EPS extraction and analysis methods have been tested on a variety of relevant organisms being utilized in the lab including *E. coli* (strains including O157:H7, D21g, XL1, and numerous natural isolates), *Salmonella enterica* serovar pullorum *Burkholderia cepacia* G4g, and *Halomonas pacifica* g. The ability to extract and analyze the EPS of *E. coli* isolates from dairy cattle and humans, as well as the *Salmonella* strain, is being tested for cells that have been stressed through starvation (0, 6, 12, and 18 hours). Additionally, a study is ongoing looking into the influence of solution chemistry (artificial groundwater at varying ionic strengths) and exposure time on *Salmonella* EPS production.

The second area of research involves the investigation of cell adhesion and transport in groundwater environments. This work is conducted in two experimental systems: 1) a packed bed column, and 2) a radial stagnation point flow (RSPF) flow cell. Both systems simulate transport of bacteria within porous media. The packed bed column is a macroscopic approach of quantifying cell transport; whereas, the RSPF system is a microscopic method of observing cell adhesion to a surface. Experiments in both systems are conducted under solution chemistry and hydrodynamic conditions simulating the subsurface environment. Experiments in the packed bed have been conducted utilizing *E. coli* (strains including O157:H7, D21g, XL1, and numerous natural isolates) and *Salmonella enterica* serovar pullorum. *Burkholderia cepacia* G4g and *Halomonas pacifica* g have been used in the RSPF system. Both methods allow for a quantification of cell transport. To fully analyze the trends in cell transport, the surface chemistry of the bacterium requires consideration. The EPS composition and content provides considerable insight into this surface chemistry. Hence, EPS analysis (as described above) has been compared to the transport data. To date the trend observed, regardless of cell type or environmental condition, is that the charge on the cell surface (as determined through measurement of zeta potential) and the ratio of sugars to proteins within the EPS provides an indication of the type of interaction forces that will result between the cells and the surfaces (aquifer sand in the column or quartz surface in the RSPF). Ongoing work will provide further insight into the applicability of this trend and whether this may provide a future predictive tool.

The overall goal for this project is that this fundamental research will provide a greater understanding of how environmental conditions influence cell fate in groundwater; and hence, lead to more effective water management and re-use practices in the future.

Publications

Haznedaroglu, B., Bolster, C.H., and S. L. Walker "The role of starvation on bacterial adhesion and transport in saturated porous media" *Water Research* (2008) 42:1547-1554

Bolster, C.H., Haznedaroglu, B., and Walker, S. L. "Diversity in cell properties and transport behavior among 12 environmental *Escherichia coli* isolates" *Journal of Environmental Quality* (2008, in press)

Selected Professional Presentations

Chen, G., Beving, D.E., Bedi, R.S., Yan, Y. and Walker, S.L. "The Antifouling Effect of Zeolite Surfaces on Bacterial Deposition in a Parallel Plate Flow Cell" American Chemical Society 82nd Colloid & Surface Science Symposium, June 15-18, 2008, Raleigh, NC.

Gong, A.S., Benavides, M., and Walker, S.L. "Extraction and Analysis of Extracellular Polymeric Substances: Comparison of Methods and EPS Levels in Salmonella sp." 108^{th} General Meeting of the American Society for Microbiology, June 1-5, 2008, Boston, MA

Haznedaroglu, B.Z. and S.L. Walker "Establishing the influence of starvation upon the transport of environmental *Escherichia coli* isolates" Joint American Chemical Society and American Institute of Chemical Engineers Spring Meeting, April 6-10, 2008, New Orleans, LA.

Collaborative Efforts

This effort has been in compliment to a National Water Research Institute (NWRI) grant which ended in October 2007. The NWRI grant's objective is also to identify the role of cell surface polymers on bacterial fate and transport. The combination of the Center for Water Resources and NWRI funds allowed for the full support of a graduate student, an undergraduate student researcher, and related experimental and travel expenses.

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