

Final Report – Ferrographic Tracking of Bacterial Transport

The work performed during the past three years has been extremely productive. Ferrographic capture was utilized in analysis of several thousand field samples collected from arrays of multilevel samplers during three intensive field campaigns conducted at two shallow sandy aquifer sites in Oyster, VA. This work has shown resulted in three important conclusions:

- 1) Ferrographic capture provides unparalleled low quantitation limits for bacterial cell enumeration (Johnson et al., 2000).
- 2) The high-resolution analyses provided by ferrographic capture allowed observation of increased bacterial removal rates (from groundwater) that corresponded to increased populations of protozoa in the groundwater (Zhang et al., 2001). This novel data allowed determination of bacterial predation rates by protists in the field, a consideration that will be important for successful bioaugmentation strategies.
- 3) The high-resolution analyses provided by ferrographic capture allowed observation of detachment of indigenous cells in response to breakthrough of injected cells in groundwater (Johnson et al., 2001). The implication of this unique observation is that bacterial transport, specifically bacterial attachment and detachment, may be much more dynamic than has been indicated by short-term laboratory and field studies. Dynamic attachment and detachment of bacteria in groundwater may lead to greatly increased transport distances over long terms relative to what has been indicated by short-term laboratory and field studies.

During September, 1999, the ability of ferrographic tracking to enumerate bacterial concentrations in aqueous suspensions of strain DA001 was compared to that of several other conventional and innovative tracking methods in the context of a laboratory column bacterial transport experiment. The other methods employed included direct counts (the bacterium was CFDA-stained), culturing, quantitative PCR, flow cytometry, and ^{13}C . All of the methods agreed within a factor of two or three for the majority of the bacterial concentrations eluted from the column. This agreement between the methods was highly encouraging considering that the cell concentrations ranged from a few to nearly $1\text{e}8$ cells/mL.

Ferrographic tracking was employed as one of several methods utilized in bacterial tracking in the field during an experiment performed at the Narrow Channel site at the South Oyster study area during October, 1999. The transport experiment involved collecting samples from 24 wells (samplers) over six different discreet depths (ports). Over 3000 samples were collected for each of the tracking methods, and about 1500 samples were analyzed by ferrography. Ferrographic tracking provided high-resolution data that spanned a range of 8 orders of magnitude in concentration. It was clearly observed from the data that the breakthrough concentration was generally highest in a given port for wells located on the major flow axis and closest to the injection well. The effect of cross-contamination in the vacuum manifolds (which provided suction to bring water up to the surface from all ports) was observed by the increase in cell counts in all ports following breakthrough in any port. The cross-contamination was not laboratory

contamination since standards were not affected. The breakthrough of the bacterium clearly indicated that heterogeneity in the vertical dimension at this site greatly affected bacterial transport velocities.

Continuous long-term collection of samples Narrow Channel (by Keun-Hyung Choi and Fred Dobbs at Old Dominion University) provided the opportunity for ferrographic analysis to monitor the low concentrations of bacteria which detach into the aqueous phase long after the injection pulse has passed. It was observed that rates of bacterial loss from the aqueous phase increased as the groundwater velocity decreased during return to natural gradient conditions. This is opposite to what was expected based on filtration theory. However, it was determined that the increase in bacterial loss rates was coincident with blooms of protozoa that grazed on the injected bacteria. Hence the bacterial loss rates were found to represent predation rather than attachment.

Use of FITC-conjugated antibody during ferrographic capture allowed both the injected and background bacteria that display similar antigens as DA001 to be enumerated. The difference between bacterial counts using FITC-conjugated versus non-conjugated antibodies (using CFDA stain for identification) allows an assessment of the background concentrations of DA001-like bacteria. The data indicates that background and injected cell counts rose simultaneously during breakthrough of the injected bacteria.

The implication of the above unique observation would be that bacterial transport, specifically bacterial attachment and detachment, may be much more dynamic than has been indicated by short-term laboratory and field studies. Dynamic attachment and detachment of bacteria in groundwater may lead to greatly increased transport distances over long terms relative to what has been indicated by short-term laboratory and field studies.

During previous studies conducted July, 2000 at the South Oyster (SO) focus area, two strains of bacteria were injected that had been previously isolated from the subsurface of the South Oyster site. The isolates were stained with a vital fluorescent stain and injected into established flow cells at the site. Injected cells were distinguished from resident cells (unstained indigenous cells) on the basis of their internal stain. Ephemeral increases in concentrations of unstained bacteria coincident with arrival of the stained bacteria were observed in several wells during the transport experiment. The unstained cells could have originated from either the injected cell population or the population indigenous to the aquifer (not injected). To originate from the injected cell population, the unstained cells would need to have been selectively concentrated relative to stained cells during transport. This could have occurred by growth of injected cells (assuming lack of stain transfer to daughter cells), loss of stain by diffusion during transport, and lesser adhesion of unstained relative to stained cells. Cell division and stain loss in samples collected during peak breakthrough were insufficient to explain the observed pulses of unstained cells. Standard adhesion assays indicated no difference in adhesion of stained versus unstained cells. Furthermore, to explain the observed ephemeral dominance of unstained cells, selective concentration would need to have occurred exclusively on the low-concentration fringes of the bacterial plume.

Based on the above observation, it was tentatively concluded that the unstained cells originated from the cell population indigenous to the aquifer (not injected) (Johnson et al., 2001). Potential mechanisms of appearance of unstained indigenous cells include growth or detachment in response to the arrival of injected cells, with detachment more likely given the lack of a lag time between arrival of the unstained and injected cells.

A significant drawback of the July 2000 experiment was reliance on indirect means to quantify unstained cells, i.e. unstained cells were quantified by the difference between stained and total cells (stained plus unstained).

The goal of the final experiment (July 2001) was also to monitor potential enhanced detachment of cells from the site sediment (in response to the arrival of newly injected cells). In contrast to the previous field experiment, the attached cells were stained. Hence, enhanced detachment (increased resident cell concentrations in groundwater) could be monitored directly.

It was determined in July 2001 that previously injected cells (remaining from the July 2000 injection) remained intact and visibly stained in the site groundwater. The presence of aqueous July 2000 cells indicated that July 2000 cells also resided on the sediment. For the July 2001 and July 2002 experiments, the same two bacterial strains were injected, however, in July 2002 the cells were stained oppositely from the cells injected in the July 2000 injection. Switching the stains allowed for direct observation of the stained cells remaining from the July 2000 injection (hereafter referred to as stained resident cells).

Analyses of samples from the July 2001 injection experiment showed no significant enhanced detachment of stained resident cells in response to breakthrough of injected cells. Likewise, supporting laboratory experiments also indicated no significant enhanced detachment of stained resident cells in response to breakthrough of injected cells. These field and laboratory results indicate one of the two following possibilities: 1) that attached resident concentrations of stained cells were insufficient to support observation of a detachment response; 2) that enhanced detachment in response to arrival of mobile bacteria does not occur to any significant extent.

Notably, ephemeral increases in unstained cell concentrations were again observed to be coincident with breakthrough of injected cells in the 2001 experiment. However, the lack of enhanced detachment of stained resident cells in the field and laboratory strongly suggests that our original tentative conclusion that hydrodynamic collision between injected mobile and indigenous attached bacteria caused enhanced detachment of unstained cells, was incorrect. Instead, the possibility (initially considered unlikely) that stain loss from stained injected cells, by cell division or stain diffusion specifically at the low concentration fringe of the plume, must be re-examined.

The ephemeral increases in unstained cell concentrations occurred solely in shallow ports. Notably, samples from shallow ports (solely from shallow ports) showed cells

with only partial staining, with the stain occurring at only one pole of the cell, suggestive of stain loss by cell division. The fact that both of these phenomena occur solely in shallow ports may link them together. It seems reasonable to suggest that cell division in the shallow ports (at the top fringe of the injected plume) caused the ephemeral increases in unstained cell concentrations that were coincident with breakthrough of the stained injected cells.

The work has so far resulted in five published peer-reviewed journal articles from the P.I.'s group, and one additional submitted manuscript (from the P.I.'s group). The manuscripts and presentations are listed below.

The work to date has resulted in one published P.I.-led peer-reviewed journal article, two additional P.I.-led manuscripts submitted for review, and two additional manuscripts listing the P.I. as a co-author. Five presentations have been made regarding this work at scientific meetings. The manuscripts and presentations are listed below.

Students trained:

Two graduate students accomplished their degrees as a result of this project. One Ph.D. student (Pengfei Zhang) received his Ph.D. in Fall 2001, and has since that time worked as a post-doctoral researcher at New Mexico Tech. and the University of Utah. Dr. Zhang is now an assistant professor at the University of West Florida. An M.S. candidate (William McIntosh) received his M.S. degree in Spring 2002, and is now seeking employment in his home state of Georgia.

Manuscripts:

W.P. Johnson & W.O. McIntosh, **2002**, Field investigation of enhanced detachment of attached bacterial cells by hydrodynamic collision with injected bacteria, submitted to *Journal of Microbial Methods*.

W.P. Johnson, P. Zhang, P.M. Gardner, M.E. Fuller, and M.F. DeFlaun, **2001**, Monitoring the Response of Indigenous Bacteria to the Arrival of Injected Bacteria using Ferrographic Capture, *Applied and Environmental Microbiology*, 67(10), 4908-4913.

Zhang, P., Johnson, W.P., Scheibe, T.D., Choi, K., and Dobbs, F.G., **2001**, Extended Tailing of Bacterial Concentrations at the Narrow Channel Site, Oyster, VA, *Water Resources Research*, 37(11), 2687-2698.

DeFlaun, M.F.; Fuller, M.E.; Johnson, W.P.; Zhang, P.; Mailloux, B.J.; Onstott, T.C.; Holben W.; Balkwill, D.; and White, D., **2001**, Comparison of Innovative Methods for Monitoring Bacterial Transport, *J. Microbial Methods*, 47, 219-231.

Fuller, M.E.; Mailloux, B.J.; Zhang, P.; Streger, S.; Hall, J.; Vainberg, S.; Beavis, S.; Johnson, W.P.; Onstott, T.C.; DeFlaun, **2001**, M.F. Field-scale evaluation of

CFDA/SE staining coupled with multiple detection methods for assessing the transport of bacteria in situ. FEMS *Microbiology Ecology* 37(1), p. 55-66.

Johnson, W.P., Zhang, P., Fuller, M.E., Scheibe, T.D., Mailoux, B.J., Onstott, T.C., DeFlaun, M.F., Hubbard, S.S., Radtke, J., Kovacik, W.P., and Holben, W., **2000**, Ferrographic Tracking of Bacterial Transport in the Field at the Narrow Channel Focus Area, Oyster, VA, *Environmental Science & Technology*. 35(1), 182-191.