The aims of our project have not changed significantly since the original proposal. Our primary goals were to support field experiments by screening strains of bacteria to find favorable transport characteristics among field isolates and to estimate collision efficiencies for those bacteria in typical Oyster site sediments. The data we obtained were disseminated to other members of the subprogram. For example, Tim Ginn of PNL incorporated our results into his field model; Aaron Mills used our work for comparison purposes; and John Wilson used our results to determine if there is a correlation between facies type and cell adhesion. Copies of all information were also sent to Mary DeFlaun of Envirogen for incorporation into the Sample Tables. In addition to the originally proposed work, we performed longer column studies, examining the effects of aluminum, iron, and water chemistry on bacterial transport, and beginning to understand the role of electrostatic interactions as determinants of biocolloid/collector affinity.

Listed below are the milestones achieved. Brief descriptions are provided for projects done in collaboration with members of the Subprogram. Papers, which have been submitted for peer review, are attached that describe our work in more detail.

1. Transport screening experiments involving bacterial isolates from the Oyster site were performed (Table 1). These experiments were conducted in MARK columns with artificial ground water and Oyster sediments. The organisms exhibited order of magnitude differences in their affinities for Oyster sediments, suggesting that results of the field experiment should be highly dependent on the selection of microorganisms. Note that only average affinity information is provided in Table 1. We continue to find that $\alpha$, the collector affinity, is a function of depth within the MARK columns. Data for all isolates are not included in this summary, however, all data for each isolate were forwarded to Tim Ginn. These data include average $\alpha$ as a function of sediment depth, and $C/C_0$ vs. depth, in addition to velocity, pore volume, sediment size distribution, and column dimension data. We worked with sediments from the Oyster site and an artificial ground water that was designed on the basis of Oyster water quality measurements.

2. Using the most promising isolates, PL2W21 and PL2W31, MARK experiments were performed using borosilicate glass beads, quartz, and iron coated quartz for comparison. Artificial ground water was the mobile phase in all cases. As shown in Figure 1, the effective $\alpha$ was highly dependent on the collector material. PL2W31 exhibited less affinity for the sediment than for glass, quartz or iron-coated quartz.

3. We performed longer column experiments to verify the MARK column results and to more effectively compare our data to results obtained at the University of Virginia. These experiments were done in 30 cm with artificial groundwater, sediment, and bacterial strain PL2W31, the strain used for the field injects. Figure 2 shows concentration out of the column or breakthrough, and Figure 3 contains the bacterial...
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adhesion information as a function of column depth. Our data was compared with similar studies performed at the University of Virginia. Together we determined the total amount of bacteria that was injected in the field study.

4. In addition, we performed various experiments to determine the transport properties and electrophoretic mobility of PL2W31 as a function of growth media. The experiments were done in MARK columns with Oyster sediment in artificial groundwater. Cells were grown in both acetate and glucose and we found that adhesion of PL2W31 was not a function of growth media. We determined that the electrophoretic mobility of this strain grown in acetate was \(-0.423 \pm 0.007 \, \mu m \, cm/V \, s\) and for cells grown in glucose it was \(-0.419 \pm 0.029 \, \mu m \, cm/V \, s\). In conclusion we determined that growth media was not shown to affect either the affinity of the bacteria for the Oyster sediment or the electrophoretic mobility.

5. The final project we did in collaboration with other members of the Subprogram involved determining how bacterial transport varies with sediment type. We performed about 100 different MARK tests using PL2W31, artificial ground water and various facies samples obtained from the borrow pit. The results of the percent adhesion versus sample number are given in Table 2. These results were forwarded to members of the subprogram for correlation to particle size and iron content information.

6. During the course of the project, we did a significant amount of laboratory experiments that were in direct support of the field work that lead to publications. A list of the projects is as follows, the papers for each project are attached.

   a. We demonstrated the advantages of using capillary electrophoresis to determine electrophoretic mobilities of biological particles compared to other methods of determining mobilities. (Paper - Glynn, J.R. et al. "Capillary Electrophoresis Measurements of Electrophoretic Mobility for Colloidal Particles of Biological Interest", *Applied Environmental Microbiology*, under review.)

   b. We determined that \(\alpha\) is a function of depth within a saturated porous media for monodisperse, monoclonal suspensions of bacteria, and that a biomodal probability density function satisfactorily represents the \(\alpha\)-distribution. Furthermore, the form of the distribution function was supported by capillary electrophoresis measurements. (Paper - Baygents, J.B. et al. “Variation of Surface Charge Density in Monoclonal Bacterial Populations: Implications for Transport Through Porous Media”, *Environmental Science and Technology*, in press.)


   d. We examined the effects of Al-oxide on bacterial transport. (Thombre et al., “the Effects of Al-oxide coatings on the retention of bacteria in porous media” In progress.)

7. Numerous oral presentations have been or will be made based on this research. A list follows.

   a. J. Glynn, R. Arnold, K. Ogden, and J. Baygents "Electrokinetic Characterization of Monoclonal Bacterial Populations Via Capillary Electrophoresis" AIChE meeting, Miami Beach, FL 11/95.
b. B. Logan, K. Ogden, J. Baygents, Y. Sun, T. Martin, J. Glynn, and R. Arnold
"Microbial and Chemical Determinants of Bacterial Mobility in Porous Media" ASM
c. T. Martin "Effect of Fe-oxide on Bacterial Transport in Porous Media" Department
of Chemical and Environmental Engineering, University of Arizona 3/95.
d. J. Glynn "Electrokinetic Characterization of Monoclonal Bacterial Populations Via
Capillary Electrophoresis" Department of Chemical and Environmental Engineering,
University of Arizona 3/95.
e. J. Glynn, R. Arnold, K. Ogden, and J. Baygents "Electrokinetic Characterization of
Monoclonal Bacterial Populations Via Capillary Electrophoresis" World Congress,
San Diego, CA 6/95.

8. This project has been used to fund 1 Ph. D. student, 4 MS students, and 3
undergraduate students.

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and opinions of authors expressed herein do not necessarily state or reflect those of the
United States Government or any agency thereof.
Table 1. Effective $\alpha$ for Oyster Isolates

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Figure 1. Alpha as a function of collector surface for bacterial strain PL2W31.
Figure: Breakthrough curve for PL2W31 in AGW-2. The 30-cm column reactor was packed with washed, repacked Oyster sediment.
Figure: Depth-dependent calculation of $\alpha$ (collision efficiency) for PL2W31 in a 32-cm column reactor that was packed with washed, homogenized Oyster sediments. The mobile phase was AGW-2.