DOE/ER/61200--3

PROJECT COMPLETION REPORT

PROJECT TITLE: Transport of Subsurface Bacteria in Porous Media

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RECIPIENT ORGANIZATION

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Scientific Objectives

Our primary objectives under terms of the Department of Energy contract were to develop tools with which to measure the advective transport of microorganisms through porous media. Those tools were then applied to investigate the sorptive properties of representative microorganisms that were selected more or less at random from the Department's deep subsurface collection of bacteria that is maintained at Florida State University by Dr. David Balkwill. The transport screening procedure that arose from this study was also used to investigate biological factors that affect the transport/sorption of biocolloids during their movement through porous media with the bulk advective flow. Considerable effort was undertaken to provide assurances that the procedure yields results that are compatible with those of more time-honored or scientifically accepted procedures.

The nature of other methods for estimating bacterial affinity for collector surfaces is such that only microbes with relatively high affinities for the surfaces in question can be meaningfully measured without going to extraordinary lengths. Since it was hypothesized that the presence of microbes in the deep subsurface is related to their ability to avoid sorptive removal during the bulk advective transport of the mobile or carrier phase through porous saturated sediments, it became imperative to develop transport-screening procedures that produce quantitative estimates of stickiness among bacteria with low affinities for the collector. The number of independent variables in such experiments -- those resulting from variation in the natures of the collector and bacterial surfaces as well as the composition of the mobile phase -- suggest that any screening tool for establishing the determinants of biocolloid/collector affinity must be both accurate and convenient to use.

Development of the MARK method as a tool for establishing biocolloid/collector affinity.

Details of the MARK (microbe and radiolabel kinesis) method for quantitatively estimating the affinity of microorganisms for specific collectors are provided in the first of five attached manuscripts that arose out of work under the terms of our contract with the Department of Energy. In brief, the method is designed to overcome the difficulties that are inherent in virtually all alternative screening techniques by measuring the numbers of bacteria that are **retained** during passage through porous media, as opposed to those that escape sorption and are therefore detectable in the reactor effluent. The technique makes it possible to quantify the retention and hence the stickiness of poorly retained bacteria, which had previously depended upon accurately differentiating between very similar cell numbers in the reactor influent and effluent. In addition, the accuracy of cell counting in the MARK procedure was greatly improved by relying upon a radiolabeling procedure in which tritiated leucine is incorporated into cell mass during growth.

Per our attached manuscript (accepted for publication in *Water Research*) results of the MARK method have proven to be insensitive to variation in specific test procedures including the number of microbes or test volume applied, the volume of rinse solution and the velocity at which the test and rinse solutions are applied. The results of sensitivity analyses in these

stickiness to well characterized porous media. The entire test can be carried out in a two-day period at a cost in materials of less than $20. By varying the selection of media, microorganism and the ionic composition of the mobile (aqueous) phase, it should be possible to conveniently test hypotheses regarding the most important environmental determinants of cell stickiness.

Application of the MARK procedure.

In the MARK method and most related procedures, cell stickiness or affinity for the collector surface is measured in terms of collision efficiency -- the fraction of the predicted encounters between the biocolloid and the collector surface that result in sorption. The MARK method is sufficiently accurate and sensitive to support meaningful estimation of bacterial collision efficiencies as low as 2 in 100,000. We have as yet found no bacteria with collision efficiencies below 1 in 1000.

Although the number of bacteria and media types that have been screened using the MARK procedure is still small, results to date indicate that an appreciable fraction of the isolates from the DOE/Florida State University collection have unusually low affinities for the glass collector material used in the original test procedure. Results are far too uncertain, however, to risk making statements about the origin of bacteria in the deep subsurface environment on this basis.

Results of experiments designed to examine the response of the MARK procedure to changes in the ionic composition of the aqueous phase were qualitatively similar to those generated using alternative, scientifically accepted procedures. It is suggested that the MARK procedure offers a versatile, low-cost, and relatively rapid alternative to widely used procedures for estimating bacterial stickiness during flow through porous media.

Depth-dependent variation in cell stickiness in the MARK reactors.

As originally configured, the MARK method measured retained cells in the top centimeter of a cylindrical reactor that was filled uniformly with 40-micron glass beads. We were pleasantly surprised to find that the procedure is sufficiently sensitive to permit accurate measurement of bacterial stickiness to the glass medium using only millimeter sections of the column. Thereafter it was possible to measure the vertical distribution of collision efficiency in single MARK columns by sectioning the columns in millimeter slices prior to measurement of retained label. The results of this work indicate that the average or population collision efficiency decreases with depth in the column. A detailed set of experimental results is provided in the attached manuscript from *FEMS Microbiology Letters* (1994). This variation could not be explained on the basis of depth-dependent changes in bacterial dimension (bacterial size distribution was essentially unchanged during passage through the MARK reactor), suggesting that collision efficiency, or affinity for the collector is actually distributed in the original bacterial population.

This observation may have important implications for prediction of microbial transport through porous media and, therefore, for the transport of pathogens and/or microorganisms with specific metabolic or bioremediative traits in groundwater. It is possible, for example, that estimates of bacterial stickiness are consistently overestimated in tests that involve short columns, in which retention involves primarily the stickier representatives of the population as a whole. Residual microorganisms could be considerably more capable of transport over long distances through porous media. Because of the enormous reproductive capacity of bacteria, the long-distance transport of just a few cells would be sufficient to reestablish the population in a new, geographically removed area. The implications for prediction of pathogen transport are readily apparent. Similarly, it may be easier to introduce microorganisms into sediments for groundwater remediation than was once supposed.

Development of probability density functions governing distributed collision efficiency.

Given that biocolloid/collector collision efficiency is actually a variable in a bacterial population (and one whose distribution changes with distance traversed through porous media) accurate prediction of bacterial transport over long distances underground may depend on the rational mathematical representation of the probability density function that governs cell-collector interactions. To test this hypothesis, MARK column slicing experiments were used to generate depth-dependent estimates of population collision efficiency. These distributions were then used to estimate parameters for a series of proposed pdf's based on minimization of squared error between predicted and measured cell-retention data. In this manner, it was determined that a bimodal distribution function has great advantage in predicting depthwise variation in the average or population collision efficiency. The exercise and results are described in detail in the accompanying manuscript that is under preparation for publication.

Ongoing Work

Research that was carried out under the terms of our recently completed grant continues under a second DOE grant that was awarded within the Subsurface Science Program. Specifically, we have been tasked to apply the MARK method for estimating the transport characteristics of select bacterial isolates obtained from local sediments at Oyster, Virginia, the site of a DOE-sponsored field-scale investigation of bacterial transport through saturated porous media. In the course of the proposed three-year study, we will also play a role in establishing the effects of mineral surface composition, pili expression by bacteria and other factors on biocolloid-collector affinity. Application of the MARK procedure and reactor is expected to greatly facilitate our efforts in this regard. Additional work will be directed toward mathematically representing biocolloid transport through porous media in a manner that will enable us to distinguish among physico-chemical and biological heterogeneities as determinants of bacterial transport characteristics under field conditions.

Students supported entirely or in part under the original DOE grant.

Name	Thesis topic or title
Doug McCaulou	The effects of temperature and motility on the advective transport of a deep subsurface bacteria through saturated sediment.
Todd Martin	Effect iron coatings on bacterial affinity for silica sand.
Yuxia Sun	Adaptation of the MARK procedure for determination of bacterial stickiness under simulated field conditions.
Arlene Little	Undergraduate researcher--thesis not required.
Brian Biesemeyer	Heterogeneity of adhesion in subsurface bacteria.
Otto Albinger	Postdoctoral student--thesis not required

Publications and presentations resulting from DOE-sponsored research as follows:

Jewett, D.G., R.C. Bales, B.E. Logan and R.G. Arnold. Comment on application of clean-bed filtration theory to bacterial deposition in porous media. Environmental Science and Technology 27: 984-985, 1993.

Logan, B.E., T.A. Hilbert and R.G. Arnold. Removal of bacteria in laboratory filters: models and experiments. Water Research 27: 955-962, 1993.

Jewett, D.G., B.E. Logan, R.G. Arnold and R.C. Bales. Quantifying bacterial transport parameters via column experiments: a sensitivity analysis. Poster session abstracts in Biodegradation: Its Role in Reducing Toxicity and Exposure to Environmental Contaminants. 1993.

Gross, M.J., R.G. Arnold, B.E. Logan, D.G. Jewett and O. Albinger. MARK: A new method for estimating bacterial transport in porous media. Poster session abstracts in Biodegradation: Its Role in Reducing Toxicity and Exposure to Environmental Contaminants. 1993.

Gross, M.J., R.G. Arnold, B.E. Logan, D.G. Jewett, and O. Albinger. MARK: A new method for estimating bacterial transport in porous media. Joint, United States-Mexico Conference on Fate, Transport and Interactions of Metals. Tucson, 1993. (poster)

Jewett, D.G., B.E. Logan, R.G. Arnold, and R.C. Bales. Quantifying bacterial transport parameters via column experiments: a sensitivity analysis. Joint, United States-Mexico Conference on the Fate, Transport and Interactions of Metals. Tucson, 1993. (poster)

Jewett, D.G., R.G. Arnold, R.C. Bales, C.P. Gerba and B.E.Logan. Laboratory determination of quantitative descriptors of bacteria and microsphere transport through porous media. AAAS 68th Annual Meeting, Southwestern and Rocky Mountain Division. 1992.

Hilbert, T., B.E. Logan, and R.G. Arnold. A method to evaluate parameters that affect bacterial transport through porous media. AAAS 68th Annual Meeting, Southwestern and Rocky Mountain Division. 1992.

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