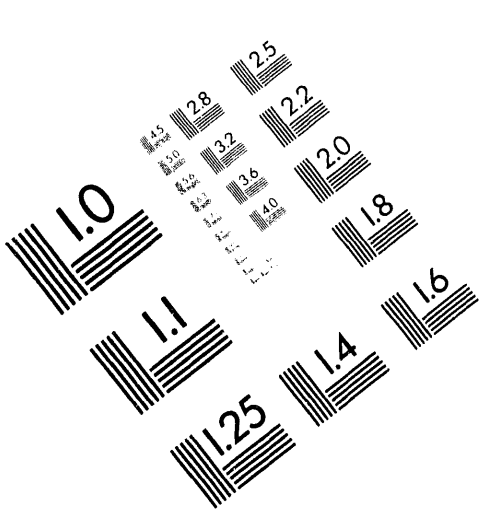

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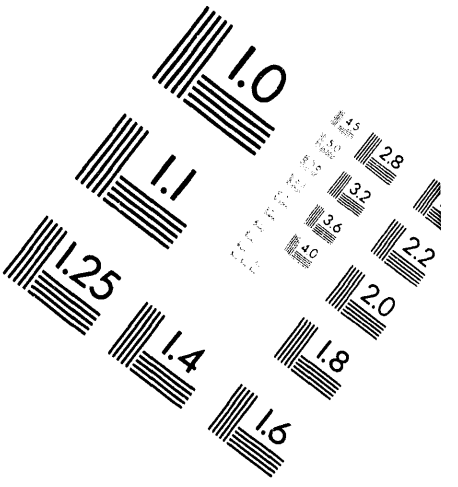

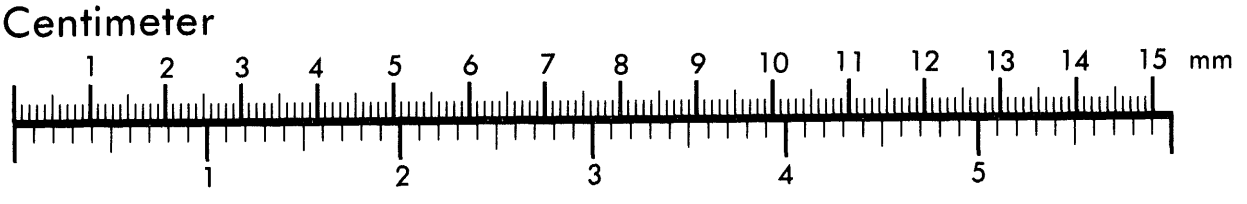


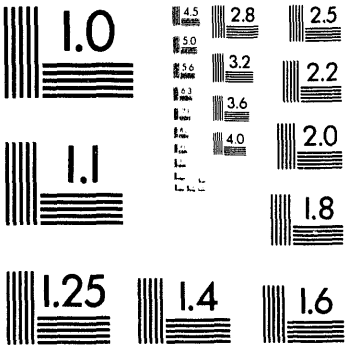

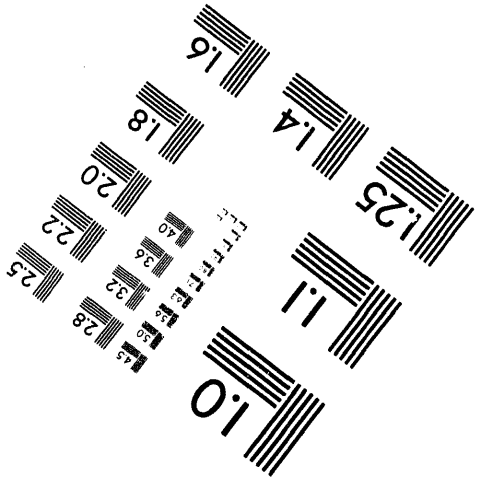

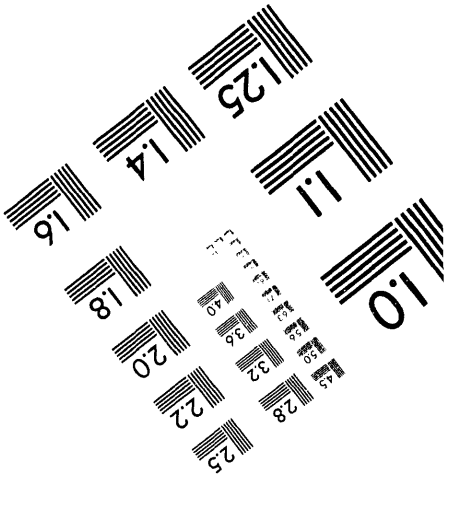

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**Progress Report, 3rd Year Continuation Proposal, and Work Plan**
Submitted to the Subsurface Science Program
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# Small Scale Laboratory Studies of Flow and Transport Phenomena in Pores And Fractures: Phase II

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**Abstract:**

Small scale laboratory experiments, equipped with an ability to actually observe behavior on the pore level using microscopy, provide an economical and easily understood scientific tool to help us validate concepts and assumptions about the transport of contaminants, and offers the propensity to discover heretofore unrecognized phenomena or behavior The main technique employs etched glass micromodels, composed of two etched glass plates, sintered together, to form a two dimensional network of three dimensional pores. Flow and transport behavior is observed on a pore or pore network level, and recorded on film and video tape. This technique is coupled with related column studies. These techniques have been used to study multiphase flow, colloid transport and most recently bacteria transport.

The project has recently moved to the Bacteria Transport Subprogram, and efforts have been redirected to support that Subprogram and its collaborative field experiment. We proposed to study bacteria transport factors of relevance to the field experiment, using micromodels and other laboratory techniques. Factors that may be addressed include bacteria characteristics (eg, hydrophobicity), pore size and shape, permeability heterogeneity, surface chemistry (eg, iron oxide coatings), surface chemistry heterogeneity, active versus resting cell bacteria, and mixed bacteria populations. In other work we will continue to examine the effects of fluid-fluid interfaces on bacteria transport, and develop a new assay for bacteria hydrophobicity. Finally we will collaborate on characterization of the field site, and the design, operation, and interpretation of the field experiment.

**Background:**

This project has been concerned with the laboratory investigation of fluid flow and transport phenomena in porous media, with an emphasis on the *in situ* visualization of the phenomena as recorded on film and video tape (despite the project title, we have not looked at fracture flow and transport issues, and have no plans to do so in the near future). The spatial scale of our efforts extends from the pore level, up to a porous media network of pores. We have evolved through three foci. We started with a focus on multiphase flow over four years ago, as part of the Multiphase Flow Subprogram of the Subsurface Science Program. Three years ago we added colloids to the multiphase flow systems, and two years ago we began to look at bacteria transport and colonization, within the same context. The goal of the project is to provide scientists and engineers with a better understanding of these transport processes. We've just moved to the Bacterial Transport Subprogram, and consequently we have dropped the emphasis on mul-

tiphase flow, and focused instead on bacteria transport. In collaboration with other Subprogram investigators, our scale of effort has grown to include the field scale. We are participating in a field investigation of bacteria transport at the Osyter Site in Virginia. Some highlights from past work in all three areas is outlined below. Current and proposed work in the bacteria transport arrea is described afterwards. Joining the new Subprogram has significantly redirected our effort, drawing us out of the laboratory into the field. The project title appears to mislable our current emphasis.

Multiphase Flow

- An experimental approach using image analysis and micromodels has been used to qualitatively study three fluid-phase displacement processes in porous media. The experiments pointed out a number of physical processes that influence the fluid distribution and saturations. Some of these processes include history and time dependent fluid flow parameters, and the drainage and imbibition of more wetting fluids via film flow. These observations suggest specific physical processes to be incorporated into percolation network and other mathematical models of pore-pressure saturation behavior. (Soll et al, 1992, 1993).
- Columns and micromodels were used to test the effects of altering the wettability of porous media on the pore pressure saturation behavior. This work tested the hypothesis that this behavior can be scaled from experiments conducted under water wet conditions. We found that scaling failed to work well, for intermediate wet conditions with strong contact angle hysteresis. There are several possible explanations for this, including the overly simplistic geometry of the scaling model, and its ignorance of water trapped in corners and wedges of the pore space. (Burck et al., 1992; see also Desai et al, 1992, & Demond et al., 1992)
- We used micromodels to investigate the different behavior of spreading and non-spreading non-aqueous phase liquids (NAPL's) under vadose zone conditions. The experiments confirmed that a positive spreading coefficient leads to NAPL that tends to spread out as a film along the gas-water interface. A negative spreading coefficient leads to a coalescence of the NAPL into isolated pockets and tiny lenses that float at the gas-water interface. This leads to an effectively lower capillary force and increases the propensity for gravity fingering. Since many common DNAPL's (dense NAPL's) are also non-spreading this indicates that they may travel along very narrow fingers vertically through the saturated zone. (Wilson, 1992)
- Aquifers contaminated with NAPL's contain liquid-liquid interfaces. The residual capillary trapped NAPL blobs are analogous to the gas bubbles of our colloid experiments (see below). Although the interfacial force balances will be different, the gas bubble results suggest that colloids will be attracted to this interface as well. This includes clays and oxides that normally coat the pore walls. We used micromodels and columns to discover that the redeposition of these particles restructured the pore space. It may also alter interfacial properties. Both mechanisms lead to a change in behavior of the non-aqueous liquid, particularly pore pressure- saturation- permeability relationships, and the efficacy of various aquifer remediation techniques. (Mace and Wilson, 1992)

Colloid Transport

- We developed a method, using etched glass micromodels, to visualize the behavior of colloid particles at interfaces within pore networks. Visualization was achieved by using glass micromodels with a UV epifluorescent light and dark field microscope. This method allowed direct observation of the interactions of colloids and interfaces both globally throughout the network and locally within individual pores. Besides fluorescent latex particles, common natural colloids, such as clay and bacteria were directly observable. The wettability of the pore walls, the fluid phase distribution within the pore network, the flow rate, and the chemical conditions of the system could all be closely controlled. Thus, the method reveals both cause and effect. This method can be used in future studies of particulate transport, interface-related transport, and the facilitation of bioremediation schemes. (Wan and Wilson, 1994a)
- Using the visualization methods and column studies we isolated several phenomena in our interface-related colloid transport study. The gas-water interface preferentially sorbs colloidal particles relative to the solid-water interface under simulated groundwater conditions. We use the general and somewhat vague term "sorption" to describe the adhesion or attachment of colloids onto any interface. The degree of this sorption increases with increasing particle surface hydrophobicity, and increasing solution ionic

strength. Hydrophobic particles have a strong affinity to the gas-water interface, but even hydrophilic particles sorb. Positively charged particles have greater affinity to the gas-water interface. (Wan and Wilson, 1992a,b; 1994a,b)

- The sorption at the gas-water interface is irreversible due to the capillary free energy change. Once sorbed onto the interface, few particles can be desorbed by chemistry or shear stress. (Wan and Wilson, 1994a).
- The irreversibility of sorption for all particles suggests a high sticking efficiency for all of the particles tested. With this interpretation the greater sorption of hydrophobic particles suggests a larger collision efficiency, presumably due to increased hydration (hydrophobic or structural) forces. (Wan and Wilson, 1994a,b)
- The preferential sorption of colloid particles onto the gas-water interface suggests a mechanism in vadose zone transport. A stationary gas-water interface in porous media can retard the transport of particulate contaminants. Moving interfaces, such as during infiltration, drainage or near a fluctuating water table may enhance colloid mobility. (Wan and Wilson, 1992a,b; 1994a,b)
- The effects of a capillary trapped non-wetting gas phase in laboratory and field colloid filtration experiments has not previously attracted attention. The porous media, thought to be water saturated, may actually be unsaturated and contain gas bubbles trapped as a residual phase. It has been demonstrated in this research that for relatively hydrophobic particles, even a small amount of residual gas can dramatically affect the transport. This issue has been essentially overlooked in published filtration experiments. (Wan and Wilson, 1994a,b)
- Although the column method has been commonly used in laboratory research, it has been demonstrated in our research that under accurately controlled physical and chemical conditions, a greater degree of reproducibility has been achieved than in previous research. (Wan and Wilson, 1994b)

Bacteria Transport and Colonization

- The glass micromodel technique allows us to observe the behavior of bacteria within porous networks. In particular we used it to determine the effect of the gas-water interface on the fate and transport of bacteria. This technique has the advantage of permitting direct observation of the behavior of bacteria on a pore and network scale under strictly controlled chemical and flow conditions. It has great potential to be used and further developed for additional purposes such as the optimization and monitoring of bioremediation processes, and monitoring the functional activity of bacteria. (Wan and Wilson, 1992b, 1994a; Wan et al., 1994)
- Using micromodels and column experiments we have quantified bacterial sorption as a function of saturation conditions. The retention of microorganisms by porous media is in part a function of gas saturation, due to preferential sorption onto the gas-water interface. Even relatively hydrophilic bacteria, which do not sorb onto the solid-water interface under unfavorable chemical conditions, are sorbed by the gas-water interface. The sorption appears to be due to the hydrophobic force: sorption at the gas-water interface increases with increasing bacteria hydrophobicity. It will be useful to test this hypothesis with other strains of relatively hydrophilic and hydrophobic bacteria. (Wan et al., 1994)
- The sorption onto the gas-water interface also appears to be irreversible due to capillary forces. A static gas-water interface sorbs and retains microorganisms, thereby reducing their transport. A gas-water interface with previously sorbed cells can be mobilized and redistributed by the increased shear stress; the mobilized gas-water interface may thus increase the movement of microorganisms. The gas-water interface is a significant and previously unrecognized factor governing the movement and distribution of microorganisms in the subsurface environment, with potential applications that include in-situ bioremediation, microbially enhanced oil recovery, and wastewater disposal. (Wan and Wilson, 1994a; Wan et al., 1994)

## Progress Summary: Bacteria Transport and Colonization, 5/1/93 - 4/30/94

In the reporting period work focused on three themes: 1) colloid and bacteria transport in the vicinity of multi-fluid phase (mostly air-water) interfaces, 2) bacteria colonization near a dissolving pool of non-aqueous phase liquid, and 3) the development of new contact angle methods for the characteriza-

tion of bacteria hydrophobicity. The work is described below. Work in area 1 has mainly concentrated on data analysis and completion of papers for peer reviewed publications. Three papers were published in this period (Wan and Wilson, 1994a,b; Wan et al., 1994; both were reviewed earlier). Work in area 2 has focused on experimental design and trouble-shooting, and collecting data. A manuscript for a peer reviewed journal is in preparation (Nowicki and Wilson, 1994). Work in area 3 has focused on exploratory investigations of alternative methods for contact angle measurement, resulting in preliminary methods refinement. In addition, a lengthy manuscript describing the overall visualization approach using micromodels was published (Wilson, 1994c) and presented in an hour long keynote address at the European Conference on Transport and Reactive Processes in Aquifers (Wilson, 1994b). A review of project work was also given as the keynote address to the U.S. Geological Survey Toxic Substances Hydrology Technical Meeting, in Colorado Springs Colorado (Wilson, 1993b), at several Universities (eg, Wilson, 1993c, 1994a), and broadcast via satellite (Wilson, 1993a). A variety of other invited talks were given at national meetings (eg, Wilson, Wan and Nowicki, 1993; Wan and Wilson, 1993).

Colloid and bacteria transport in the vicinity of air water interfaces

The major findings of this effort are summarized above; we'll amplify some of these findings here. In single phase flow experiments the behavior of abiotic and resting state biotic colloids is consistent with DLVO theory (accounting for electrostatic and van der Waals forces). The major new observation is the effect of hydrophobic (structural) and capillary forces attracting and holding particles, including bacteria, at fluid–fluid interfaces (Wan and Wilson, 1994a,b; Wan et al., 1994). Capillary forces are particularly important in holding particles on the fluid–fluid interface once they become attached (Wan and Wilson, 1994a). If a particle or bacterium with any finite contact angle touches an interface it will attach because of a capillary force. This same force is strong enough to prevent it from detaching from the fluid–fluid interface. Moving fluid–fluid interfaces, such as during wetting and drying cycle can pick up particles and bacteria and redistribute them (Wan and Wilson, 1994a; Wan et al., 1994). This phenomenon may play an important role in colloid and bacteria migration in the vadose zone.

We've observed that in a resting state the bacteria behave essentially the same as the latex particles (Wan et al., 1994). Ron Harvey of the USGS has observed differential movement of latex beads and bacteria in laboratory experiments and at Cape Cod. Our experiments clearly show that that his observation depends on experimental conditions.

The attraction of particles and bacteria to fluid–fluid interfaces is substantially greater for the more hydrophobic particles or bacteria, resulting a greater lag and attenuation of movement (Wan and Wilson, 1994b; Wan et al., 1994). The more hydrophobic the particle or bacterium the stronger the force. We've calculated the force for several geometries.

In reviewing our work several people have suggested that the retention of particles and bacteria by gas bubbles may explain some of the discrepancies in standard single phase flow colloid experiments for sticking efficiency. For example, with hydrophilic latex particles the 16% residual air saturation columns had only 91% recovery, compared to 99% for the saturated column. If some of the historical single phase flow experiments contained tapped gas they could easily lead to a miscalculated sticking efficiency (Wan and Wilson, 1994b). In reviewing the literature it is not clear that investigators have been aware of this issue, or that they took precautions to insure the absence of trapped gas.

A similar issue may be relevant to field tests, especially in shallow aquifers with fluctuating water tables. As a water table falls and then rises again, gas (air) bubbles can become entrapped beneath the water table. Colloids or bacteria moving laterally beneath the water table, in this zone of trapped gas,

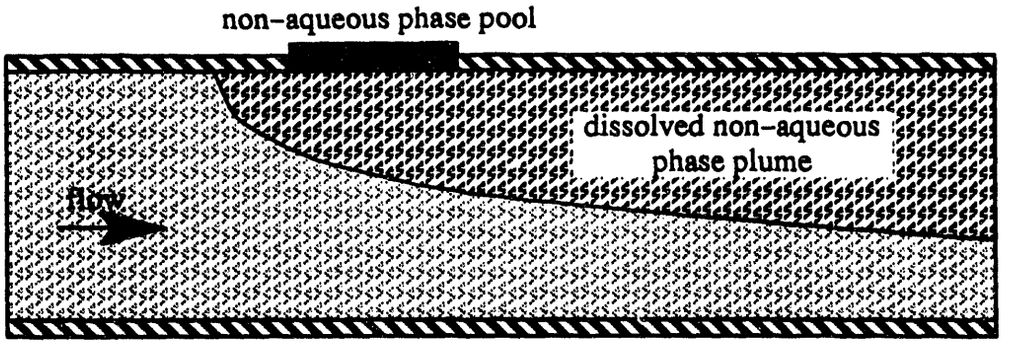


FIGURE 1 Conceptual view of Rittman dissolution experiment

may be preferentially sorbed by the bubbles. Transport experiments, such as that planned for the Oyster Site should take this possibility into account.

Bacteria colonization

Bruce Rittman of Northwestern University has been studying the bacteria enhance solubilization of a pool of non–aqueous phase liquid in flowing groundwater. We've obtained a pure culture of a toluene degrading bacteria, Pseudomonas putida PpG9, and are conducting parallel experiments in etched glass micromodels. The geometry of this experiment is pictured in Figure 1 The pool of toluene phase dissolves into the passing groundwater. The idea is that bacteria, using the dissolved non–aqueous phase as a source of carbon, lower its concentration. With a lower dissolved concentration the rate of mass transfer, and thus the rate of dissolution increases. To study this hypothesis the Rittman group has developed mathematical and sand box models of the geometry shown in the figure. Pseudomonas putida finds high concentrations of toluene toxic. Balancing the toxic effects and the carbon source it should establish itself somewhere in a mechanical dispersion dominated boundary layer region where toluene concentrations are finite but not too high.

We have reproduced their geometry in a micromodel, in order to study this spatial pattern of bacteria colonization near the pool. A diagram of the model is shown in Figure 2. The model has 0.1ml of pore volume. The model depth and model pore sizes are designed to be similar to those in the sand box. The toluene pool is contained in the very special pore at the top of the model. The connections are designed so

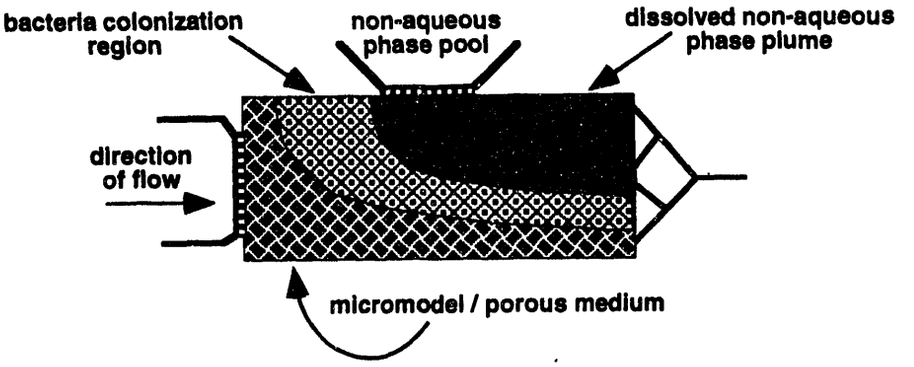


FIGURE 2 Micromodel pattern used in the dissolution experiment

that the toluene can dissolve into the model but cannot flow freely into it as a bulk phase. The flow rate is varied between 0.05ml/hr. and 6.1 ml/hr, in order to develop dissolved toluene plumes of different depths. At high flow rates the plume is restricted to the top of the model. Since there are only 24 pore bodies across the depth of the model, there is a maximum flow rate above which there is very little mixing in the model. At extremely low flow rates diffusion controls. Water samples are collected from the discharge end of the model, and tested for toluene concentrations using gas chromatography.

During this period the micromodel experiment has been undergoing tests and refinement. As with Rittman's sandbox, we have run a variety of abiotic experiments to fully understand the flow field and toluene plume before introducing the bacteria. In the simplest of these tests we have used toluene and measured effluent concentrations. We have also used dyes to observe the plume in situ. We then used latex particles to observe the transport of colloids in the pore space. These particles were selected to have a contact angle similar to that measured for Pseudomonas putida PpG9. Finally, we ran a suspension of resting state bacteria into the model, without toluene present. These control experiments tell us something about the attachment of bacteria to the glass in the absence of growth. In the colonization experiment a suspension of bacteria was run through the micromodel for 12 hours. The toluene was then introduced into the pore at the top of the model, and flow was restarted with suspension free water. The position of the bacteria was then observed at least once every 24 hours for a week.

We have also studied alternative methods to enhance visualization of the bacteria, including the use of fluorescent stains and anti-bodies. We have settled on staining the bacteria with CellTracker fluorescent probes (Molecular Probes). These stains are retained in living cells for several generations. They pass freely through cell membranes, but once inside the cell, they undergo a reaction producing a reaction product. The bacteria were determined to be relatively hydrophilic, with a contact angle of 34° (compare to angles measured in Wan et al., 1994). They did not sorb readily onto the glass surface at the relatively low ionic strength of the artificial groundwater used here ($5x10^{-4}$ M). The experiments are still on-going. The results have not yet been interpreted.

Bacterial hydrophobicity and contact angles

The third area of research concerns the development of improved methods for measuring contact angles for bacteria, as a measure of their hydrophobicity. This work commenced in January. Various contact angle methods are used by microbiologists to perform this measure (eg, van Oss and Gillman, 1972; Van Loosdrecht et al., 1987a; Wan et al., 1994). In one method a bacteria suspension is filtered onto a filter plate. Paper filters give much less reproducible results than gold filters. The filter cake is allowed to desiccate in air for a while, and then a pendant drop of water is brought into contact with the filter cake. As it touches the filter cake, an advancing contact angle can be measured, using a goniometer, but the angle changes quickly. The water in the drop begins to imbibe into the filter cake, and spread along the surface of the already water wet cake. The filter cake cannot be allowed to completely dry, even for abiotic colloids. It cracks. A biotic filter cake must also retain enough moisture for the bacteria to remain viable, in a resting state. A more common procedure is to make a smear of the suspension on a glass plate (van Oss and Gillman, 1972; Wan et al., 1994). The smear is allowed to dry, until it is about ready to crack. Again, pendant drop contact angles are measured with a goniometer. This method also suffers from water imbibition, and water spreading on the water wet smear.

Contact angle hysteresis is another problem. As the drop of water is withdrawn (into the pippette) the receding contact angle is less than the measured advancing angle. The difference in contact angle is referred to as contact angle hysteresis (see advancing and receding sketches in Figure 3). Rough surfaces increase contact angle hysteresis, and both the filter cake and slide smear are rough surfaces. With all of

these difficulties it is almost impossible to compare measurements made by different operators, much less different laboratories. A more reproducible method is needed.

We propose an improved method for measuring contact angles for bacteria, as an indicator of their hydrophobicity. The improved contact angle method involves only two fluids: water and oil, but no air. In this method a bacteria suspension is placed in a spectrophotometer cuvette. The cuvette has excellent optical properties, insuring that contact angles measured in situ will be accurate. The cuvette is centrifuged at standard accelerations, until a bacteria pill has been created in the bottom of the cuvette, with a solution of water above. It is then removed from the centrifuge and placed on the stage of the goniometer microscope, which provides a side view of the bacteria bed and overlying aqueous solution. A nanoliter pipette is then used to bring a drop of oil into contact with the bed, and the contact angle through the oil drop is measured.

This method avoids the water wet issue. In the standard experiment both air and water are initially present in the bacteria bed. In this experiment only water is present as the ambient fluid, and oil is used to make the measurement. This eliminates the imbibition and spreading issues. It does not eliminate the roughness issue, although the centrifuged bacteria bed is almost as smooth as any filter bed, and smoother than a smear.

Preliminary development and testing of the method has been underway for several months using polystyrene latex, as a model colloid, and several different pure cultures. No conclusions can yet be drawn from this work.

**Proposed Research: Bacteria Transport, 9/1/94 -8/31/95**

The Bacterial Transport Subprogram has a collaborative field research effort at the Oyster Experimental Site. In this experiment the Subprogram will test the hypothesis that field scale heterogeneity controls the transport of bacteria. Heterogeneity of permeability and iron oxide coatings on the mineral grains are being investigated. It is hypothesized that the permeability heterogeneity controls preferred flow paths, while iron oxide coating heterogeneity controls selected retention of microorganisms.

With the redirection of the new subprogram our third year of work will focus on bacteria transport and the support of the bacteria transport experiment at the Oyster Site.

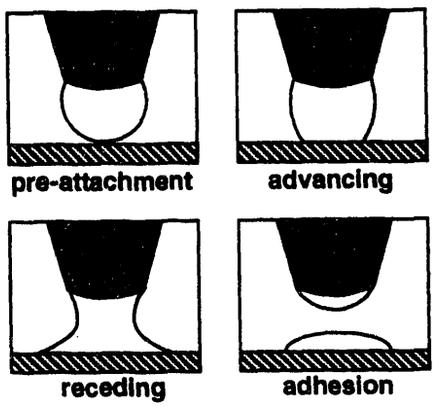


FIGURE 3 Contact Angle Measurement

Oyster field experimental work

Our work on the Oyster, Virginia field experiment has four aspects. First we will collaborate with George Hornberger and colleagues at the University of Virginia (UVa) with site characterization and the design of the experiment from both hydrologic and geostatistical perspectives. We have extensive experience in this area. We'll be in the field starting in June.

Second, under separate funding through a proposal submitted several months ago, we will work with UVa and INEL on the geological and geostatistical characterization of aquifer heterogeneities. This effort will include extensive geological facies mapping (Davis et al., 1993), and air mini-permeameter measurements of permeability (Davis et al. 1994), on a nearby contemporaneous outcrop (and possibly a more extensive, but more remote outcrop). The mapping effort will involve other collaborators, from INEL (Bob Smith, Arthur Lee) and other universities (possibly Matt Davis of the University of New Hampshire). Our our main effort will be establishing the methodology and reliability of the air mini-permeameter measurements, and the taking the measurements themselves (see separate proposal). With UVa we'll also collect samples, at the same locations, for an iron oxide assay. In the fundamental hypothesis being tested we assume that the iron oxide coatings significantly enhance bacteria attachment. The group will test for the active attachment sites using batch sulfate sorption experiments employing $S^{35}$ radio-labeled tracer. Measured values of sorption coefficient, permeability, and grain size distribution will be correlated with geology. We'll kick start this effort later this month (June) using current funding.

Later we will work with project collaborators to establish geostatistical and geologic correlations between the outcrops, cores behind the outcrop, and cores at the site, in order to link the outcrop study to the site and improve its characterization. Classical geostatistical methods will be used including variogram and cross-variogram estimation, as well as more the exotic techniques proposed by Craig Loehle of Argonne National Laboratory.

The third area of collaboration will be with Aaron Mills at the University of Virginia, Roger Bales and colleagues at the University of Arizona, and possibly others. It concerns column and visualization experiments on the transport properties of site microorganisms. In particular we expect to examine bacteria attachment and detachment for typical aquifer materials, as observed and collected in the cores and outcrop, and in the presence of aquifer hydrogeochemistry. This work is described in the next section.

The fourth area of collaboration concerns the construction and running of the field experiment. It is important that our students have exposure to the experiment itself, not just support activities. Thus we plan that they will spend at least several weeks helping the University of Virginia crew with this activity.

Bacteria transport experiments in support of the field experiment

Column and micromodel experiments will be run in support of the field work. In these water saturated experiments we will work with the University of Virginia and the University of Arizona to run micromodel experiments in collaboration with their batch and column experiments, and MARK assays. Emphasis will be placed on role of iron oxide coatings and media heterogeneity in bacteria attachment and detachment . We are currently developing methods to coat micromodels that are analogous to the methods used at the University of Virginia for their sand columns. We have already developed micromodels with heterogeneous permeability. Techniques are under investigation for building heterogeneous coatings.

Using the micromodels, and column experiments here or elsewhere, we will study the transport of bacteria though different pore geometries, surface chemistry, and heterogeneity. We'll explore the movement of pure cultures of bacteria with different hydrophobicity and other characteristics, agreed

upon by our collaborators. Besides hydrophobicity (Van Loosdrecht et al., 1987a; Bales et al., 1993; Wan et al., 1994), characteristics to be considered include surface charge (electrophoretic mobility; (Van Loosdrecht et al., 1987b); cell size, shape and morphology (Kjelleberg and Hermansson, 1984); gram+ or gram–. Pure cultures from collections within the Subprogram or isolated from the experimental site will be used.

Six different kinds of laboratory experiments will be considered to support the overall goals of the field program. Each type of experiment tests a different hypothesis.

1. The first set will be aimed at the role of pore geometry on bacteria transport. We will construct micromodels with different pore structures (see, eg, Wan and Wilson, 1994a), simulating the different textures of material encountered in the field. Preliminary characterization of grain size, shape and sorting from the outcrop suggests a strong correlation to permeability (Bob Smith, personal communication, 1994). Will preferential transport or retention of microorganisms in these texturally varying materials be due to the differences in permeability, or the differences in pore geometry and its direct effect on transport (collision efficiency and sticking efficiency) and sorption (Matthess and Pekdeger, 1985; Fates et al., 1991; Gannon et al., 1991) ?

2. Second, we'll construct micromodels with heterogeneous pore spaces, to test the control that permeability heterogeneity has on bacteria transport (Harvey, 1991). Previous column experiments have indicated early breakthrough of bacteria, when high permeability paths are connected through the sample (Saiers et al., 1993). When high permeability lenses are embedded in the sample the results are less conclusive (G. Hornberger, personal communication, 1994). Micromodels can be used to explore this issue.

3. Third, we'll study the attachment/detachment mechanisms for surfaces coated with iron oxide (Mills et al., 1993), and perhaps other common mineral coatings (Scholl et al., 1990). There is now significant evidence within the Subprogram that these coatings retard bacteria movement. We'll make our observations in a micromodel in which only the pores in the bottom plate have been coated, thus allowing direct viewing through the top plate. Among the specific hypotheses we'll test are that sorption is kinetically controlled, and attachment is reversible (Bales et al., 1993; Grant et al., 1993).

4. Fourth, we'll selectively apply the coating over the surface of the micromodel interior, simulating the small scale spatial distribution of oxides observed in cores and at the outcrop. Again, we'll observe attachment and detachment *in situ*. Here we'll test the hypothesis that heterogeneity of surface chemistry begets kinetically controlled attachment and detachment.

5. Fifth, we'll explore the difference in bacteria transport for resting cells vs active cells. The field experiment is proceeding on the assumption that the injected cells will remain in a resting state. However, it is possible that they will find sufficient energy to become more active. Using micromodels we'll begin to explore the effect on transport that this difference in state presents (size, shape, morphology, motility, charge, hydrophobicity, etc.; see Kjelleberg and Hermansson, 1984; Ford et al., 1991; Sharma and McInerney, 1994).

6. Sixth, and finally, we may explore the transport of a mixed population of resting state bacteria. In particular we'll examine competition for attachment sites for both coated and uncoated micro-

models, with and without heterogeneity. The mixed population will composed of a combination of well characterized pure strains. We will used specific fluorescent probes for some or all of the strains, allowing us to distinguish one strain from another under the epifluorescent microscope. Later we may attempt to study a consortium of bacteria from the site, where specific strains are added and stained for identification.

In all of these experiments the aqueous phase composition presents an additional degree of freedom. For most of these experiments we'll use an artificial groundwater agreed upon by the Subprogram.

These experiments are not listed in order of priority. Specific experiments to be run will be selected collaboratively with other investigators in the Subprogram, and coordinated with their laboratory experiments and the field experiment.

Bacteria transport in the vicinity of air water interfaces

Work on the attachment of bacteria to fluid-fluid interfaces will continue during this period, following two lines of inquiry. First, a two sorption-site mathematical transport model will be developed and applied to our published results for gas bubbles. The model will follow a standard advection-dispersion formulation (eg, Corapcioglu and Haridas, 1985), with two sorption sites representing the solid and gas bubbles. Equilibrium sorption modeling will be employed, using appropriate parameters for collision and sticking efficiencies. The model will be used to test our understanding of bacteria transport in the two fluid situation, where the non-wetting fluid is at residual.

The second line of inquiry will lead to a field test of the hypothesis that there is significant bacteria sorption at fluid-fluid interfaces. Utilizing the Oyster experimental site we will design a test in which groundwater controls will be used to lower the water table, and then raise it again, trapping air bubbles in the pores in the upper part of the saturated zone. Tracers, eg helium, will be used to confirm their presence. Then a bacteria transport experiment will be conducted, following the procedure used in earlier water saturated experiments, as modified by experience and additonal site characterization. The two sorption-site model will be used to assist in test design and interpretation. The test will be proposed for the second field season.

Air bubbles can also be inadvertently introduced through drilling and other activities, and purposefully introduced through air sparging. Further work will be proposed at a later time to explore whether attachment of microorganism to these bubbles is an important issue.

Bacterial hydrophobicity and contact angles

Work on the proposed improved method for measuring contact angles for bacteria, as an indicator of their hydrophobicity, will be continued. In this period the procedure will be finalized and tested in collaboration with other subprogram investigators (University of Virginia, University of Arizona). These results will be correlated with the standard air/water contact angle smear method used here and at the University of Virginia, with the standard MATH Assay approach, and with the new MARK Assay developed at the University of Arizona. The MARK Assay addresses hydrophobicity along with other effects. Once the new procedure has been stabilized and tested here, cross-laboratory tests will be made by collaborators.

In the new method various oils can be used, each with a different hydrophobicity. Contact angles on a single pure culture can be measured for a variety of oils, providing greater sensitivity of the measurement. We'll look for indices of these multiple oil tests to summarize the results more simply.

The method yields other measures of hydrophobicity, besides the contact angle. When the drop is initially brought into contact with the bacteria bed, does it spread or is it stable? When the drop is withdrawn into the pipette, does it withdraw cleanly into the pipette, or does it snap-off and leave behind a captive drop on the surface of the bed (see lower right corner of Figure 3). Coupled together with the use of oils of varying hydrophobicity, these discrete measures should provide a reproducible classification system for hydrophobicity.

The test is being developed for resting state bacteria. During this period preliminary experiments will be taken to explore its applicability to growing and perhaps motile bacteria. Bacteria hydrophobicity is a function of growth state. If this method works out, additional effort relating metabolic state to hydrophobicity may be warranted.

Experimental Approach and Methods

We currently employ three basic experimental tools in our laboratory: micromodel visualization experiments (Conrad et al., 1992; Wan and Wilson, 1994a; and Wan et al., 1994), column experiments (Wilson et al., 1990; Mace and Wilson, 1992; Wan and Wilson, 1992a,b, 1994b; Wan et al., 1994), and surface behavior experiments. The surface behavior experiments concern contact angle measurements on surfaces (Burck et al., 1992; Wei et al, 1993), colloids (Wan and Wilson, 1992b,1994a,b), and bacteria (van Oss and Gillman, 1972; Wan et al., 1994). Of course we are working on improving this method.

Etched glass micromodels are composed of a network of pore bodies connected to pore throats. The networks are only two-dimensional although the pores are three dimensional (Wilson, 1994). Fluid flow, colloid transport and bacteria behavior can be visually observed in these models using microscopy (Carl Zeiss Axiophot equipped with transmitted and reflected light; epifluoresent light; and dark field). Our conventional micromodels have pores ranging from 100 microns to a few millimeters in size, but Wan and Wilson (1994a) describe new models with pores as small as 20 microns. The smaller pore sizes are needed in our colloid and bacteria work.

To date our columns employ a high purity quartz sand (Uninin Co., Ne Canaan, CT., the same material and supplier agreed upon by the MFF subprogram) in cylindrical glass columns (ACE Glass, Inc.). We have developed careful cleaning procedures (Wan and Wilson, 1994b).

The column and micromodel bacteria experiments consist of saturating the experiments with water, then creating the desired experimental condition. For example, in the air bubble experiments the water was displaced with air Afterwards the air was displaced to residual non-wetting phase saturation by water. A slug of 1 or more pore volumes of dilute bacteria suspension was then injected. The slug was followed by a suspension free solution. These micromodels only had pore volumes of 100-500 microliters, so their slug was as large as 30 pore volumes. In the columns the effluent is collected in a fraction collector at a rate of 6 or more samples per pore volume, while in the micromodels the bacteria are observed and recorded. The column effluent concentrations are then plotted as breakthrough curves.

## Equipment

We are proposing a modest equipment budget, using cost sharing funds supplied by Tech. The money will be used to purchase a swinging buket rotor for our programable Beckman centrifuge (floor model J2-MI centrifuge with high speed, induction drive; 20,000g's). The centrifuge is used in the contact angle experiments.

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