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Processes Controlling the Migration and Biodegradation of Non-Aqueous Phase Liquids (NAPLs) within Fractured Rocks in the Vadose Zone

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FY97 Annual Report

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and Jennie C. Hunter-Cevera

Earth Sciences Division

February 1998

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**Processes Controlling the Migration and Biodegradation of
Non-aqueous Phase Liquids (NAPLs) within Fractured Rocks in the Vadose Zone
FY97 Annual Report**

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1. Executive Summary

Background

Subsurface contamination from volatile organic compounds (VOCs) has been found at many Department of Energy (DOE), Department of Defense (DoD) and industrial sites due to the widespread use of organic solvents and hydrocarbon fuels. At ambient pressures and temperatures in the shallow subsurface, these substances are liquids that are immiscible with water; hence they are commonly designated as non-aqueous phase liquids (NAPLs). At some DOE sites, NAPLs are the presumed source of groundwater contamination in fractured rocks, such as basalts (at Hanford and Idaho National Engineering and Environmental Laboratory (INEEL)), shales (Oak Ridge Y-12 Plant), and welded tuffs (Los Alamos National Laboratory (LANL)).

The flow, transport and biodegradation processes controlling NAPL behavior in the vadose zone must be understood in order to establish the possible extent of contamination, the risk to groundwater supplies, and appropriate remediation action. This is particularly important in arid sites with deep water tables (such as at Hanford, INEEL and LANL). In fractured rock aquifers, NAPL migration is likely to be dominated by the highly permeable pathways provided by rock fractures and joints. Two- and three- phase fluid phases may be present in vadose zone fractures, including NAPL-gas, NAPL-water (in regions of perched water) and NAPL-water-gas.

It is essential to determine the potential for biotransformation of contaminants in these environments, either naturally or as a result of stimulation. Fluid flow and distribution are likely to be significant factors in the ability of microorganisms to degrade NAPL contaminants, as they affect the transport and availability of substrate, moisture and nutrients. Biological activity can produce changes in liquid surface tensions and generate biofilms that may change the wettability of surfaces, locally altering fracture permeability and altering fluid flow and distribution.

Project Focus

To address the strong coupling between fluid distribution and the potential for biodegradation, this project investigates both flow dynamics and microbial processes affecting NAPLs in fractured rock in a closely coupled, integrated manner. Our objective is to develop an understanding of the behavior of two and three immiscible fluid phases, microbial transformation and/or degradation of NAPL contaminants, and to provide a scientific basis for field investigations, site characterization, and remedial action for NAPL

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contamination in fractured rocks. To achieve this, our program combines laboratory and theoretical investigations, coupled with the evaluation of conditions at relevant field sites.

Summary of FY97 accomplishments

Major FY97 accomplishments for project tasks are highlighted below. The body of this report includes a more detailed summary of our activities, and a summary of publications and conference papers that have come out of this project in FY97.

Task 1. Phenomenological studies: to explore and visualize two- and three-phase flow and microbial activity in natural rock fractures, and in transparent replicas of natural fractures.

Methodology for conducting "rock-replica geocosm" experiments to allow visualization of water seepage on a natural rock fracture (dense basalt) inoculated with micro-organisms was developed. The first experiment showed that over time the number of colony forming units in the seepage effluent increased by one order of magnitude, surface tension of the effluent water decreased by 30% and ponding of water due to local clogging of the pore-space affected seepage behavior.

Task 2. Measurement of two- and three-phase flow in fractures: to quantify the relationships between rock and fluid properties that determine (a) the spatial and temporal distribution of immiscible infiltrating liquids in fractures, and (b) the hydrogeochemical environment for microbial activity.

- Using water seepage data from a variety of flow cells (fracture replicas, rock-replica combinations and parallel glass plates of small-medium-small aperture sequences), the frequency of intermittent flow events was correlated on the basis of Capillary and Bond Numbers and shown to reveal consistent trends. A manuscript describing this work (Su et al., submitted) has been submitted for journal publication.
- Seepage experiments in a fracture replica have been conducted for liquids of varying density, viscosity and wettability. These parameters have little effect on the liquid distribution in an initially dry fracture, but they have a strong effect on the time-dependent behavior (i.e. frequency of channel snapping and reforming).
- Tracer tests in a fracture replica measured the travel time of seeping water and showed that the travel time increases at higher angles of inclination due to the increased frequency of intermittent flow events.

Task 3. Biotransformation of volatile NAPL organic contaminants: to measure the extent of biotransformation and/or degradation occurring in NAPL-contaminated fractured rock and assess monitoring tools.

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- A technique for aseptically sawing thin slices of rock core samples was developed and shown to maintain the form and arrangement of microorganisms on rock slices.
- Stand-alone and synchrotron-based IR microspectroscopy techniques were developed and tested to monitor changes in microbial activity on rock surfaces as a function of environmental conditions. In preliminary stress-response experiments, changes in the IR spectra were used to identify the response of bacteria to changing relative humidity.
- A preconcentration system for use with a new stable isotope mass spectrometer at the Center for Isotope Geochemistry at the E. O. Lawrence Berkeley National Laboratory was acquired for use with this project. This configuration will allow the measurement of isotope ratios of much smaller amounts of gas (by two to three orders of magnitude) compared to conventional dual inlet machines. The implications for the analytical support of our geocosm experiments are (1) an increased sensitivity to detect microbial metabolic activity from shifts in isotope compositions and (2) use of stable isotope labeled substrates instead of radio-labeled compounds, thereby minimizing mixed hazardous waste volume.

Task 4. Theoretical analysis: to develop and test conceptual models that encompass the physical, chemical and biological processes relevant to the fate of NAPLs in fractures.

Numerical simulation of water seepage in unsaturated fractures considered detailed resolution of heterogeneity in several ways:

- Introduction of asperity contacts, fracture terminations and subvertical heterogeneity features illustrated mechanisms for preferential fast flow in localized pathways due to partial exclusion of flow from portions of the fracture and flow funneling. These results are described in Pruess (1998).
- The use of "simulated annealing" as a more flexible approach (compared to "turning-bands method") for generating spatially correlated heterogeneous fields. The standard Metropolis algorithm was modified to treat peculiar heterogeneity features of fractures (e.g., presence of asperity contacts), and we demonstrated applications of synthetic heterogeneous fields for modeling unsaturated flow of water.

Relevance to field cases

Project participants' involvement in other projects with field activities at INEEL provides our laboratory and theoretical components with insight into field issues. Soil vapor samples collected from the vadose zone at the Radioactive Waste Management Complex (RWMC) at the INEEL have elevated concentrations of CO₂ (up to 2%) in the vicinity of pits used to dispose of lubricating oil mixed with chlorinated solvents. Isotope measurements of this CO₂ indicate that it is at least partially derived from biodegradation of

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the oil and/or chlorinated solvents, suggesting that microbial activity in the vadose zone does have a significant effect on the fate of the contaminants.

In conjunction with this project, a series of vapor samples were collected from the Test Area North (TAN) site at INEEL. The concentrations of CO₂ in these samples were less than or equal to atmospheric CO₂ concentration (~360 ppm). However, concentrations of O₂ in the samples were also depleted relative to atmospheric concentrations, indicating that microbial oxidation of organic matter is occurring in subsurface, but the resulting CO₂ is being removed from the soil vapors, possibly by absorption of the CO₂ into the slightly alkaline groundwater. Low $\delta^{13}\text{C}$ values for CO₂ (relative to atmospheric CO₂) from two of the samples provides further evidence that the CO₂ is derived from oxidation of organic matter (which has low $\delta^{13}\text{C}$ values).

These data suggest that subsurface microbial activity in the vadose zone is significant at INEEL. Future field work will concentrate on determining the source of the organic carbon in the vadose zone at the TAN site (contaminants or natural sources) and how it is being transported there (e.g., vaporization of volatile compounds, infiltration in vadose zone pore water, natural organic carbon in sedimentary interbeds).

Plans for 1998

There is a growing perception that dense non-aqueous phase liquid (DNAPL) contamination in fractured rock is an intractable problem. The current inability to locate, much less remove DNAPLs from fractured rock sites makes it essential to determine the potential for biotransformations of contaminants to non-toxic endproducts and whether these transformations can contain the spread of VOC contamination. Given the field evidence of biological activity at contaminated fractured rock sites (described in the previous section), characterizing the potential for biological transformations and limiting factors should lead to methods to stimulate biological activity.

We have found that one of the major research challenges in studying DNAPL fate and transformations that account for mass transfer limitations due to transport in a fracture (as opposed to batch studies) is to design laboratory experiments that are meaningful and relevant representations of subsurface conditions. Our work in the second half of our project will build on and further develop our tools to advance our understanding of transformations in fractured rock.

We will conduct additional rock-replica geocosm experiments with lower initial surface bacteria concentrations and supporting batch studies to compare growth with and without the mass transfer limitations induced by the flow field in the fracture plane. Flow

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dynamics experiments in single fractures with replicas and rocks, including liquid seepage with varying initial residual saturations, will be conducted. We will pursue opportunities to obtain larger fracture samples for seepage studies from INEEL this Spring and Summer. Development of Fourier transform infrared (FTIR) spectroscopic techniques will continue. This technique will be used to screen various environmental factors that affect biotransformations on fracture surfaces and to establish the conditions for the fractured rock geocosms. Theoretical studies will aim at developing conceptual and mathematical models for multiphase fluid behavior in fractured rock. As all of the researchers in this project participate in other projects with field activities, we will continue to obtain information on VOC-contaminated fractured vadose zone sites, and will refine our laboratory-scale investigations where needed to address field and monitoring issues.

2. Task 1. Phenomenological studies: open and rock-replica geocosms

Introduction

In 1997 this effort focused on developing experiments that integrate flow dynamics and biological processes in order to understand the relationship between seepage patterns and microbial activity. The hypothesis is that biological growth will most readily occur in the path of the seepage water, and will alter the transport properties of the rock due to blockage or change in solid surface properties by biofilm formation, and/or change in liquid surface tension. The presence of residual NAPL will further alter patterns of microbial activity and subsequent water seepage. Following is a brief description of the laboratory experiments; an LBNL topical report is in preparation which will include more of the experimental details.

The laboratory experiments attempt to mimic some of the essential features of natural conditions, while providing direct observation of flow dynamics. One half of a natural rock fracture is used, either as an open surface (called the open geocosm), or mated with a transparent replica of the second half of the rock fracture (called the rock-replica geocosm). Two open geocosm tests were performed, one with a dense basalt fractured core sample from Box Canyon at INEEL, the second on a larger dense basalt outcrop sample obtained from the same site. The outcrop sample was also used for the rock-replica geocosm, where one half of the rock fracture was mated to an epoxy replica cast from the second half of the fracture.

Description of Experimental Apparatus

The rock or rock-replica assembly is enclosed in a box with a glass cover, in which ambient air is maintained at 100% relative humidity, typical of the vadose zone (Figure 2.1). Relative humidity is maintained by heating a reservoir of water inside the box to a temperature just above ambient and submerging the box in a water bath chilled to several degrees below ambient temperature. Water that condenses on the inside of the box walls is collected and returned to the reservoir. The air temperature and barometric pressure inside the box are continuously monitored.

The seepage water in the open geocosm was distilled water with 1 g/L fluorescein to enable visualization of seepage patterns by illumination with near-UV light (non-biocidal) which were recorded by video photography. Except during photography, the box was dark to prevent the growth of photosynthetic organisms. In the rock-replica geocosm,

2. Task 1. Phenomenological studies: open and rock-replica geocosms

seepage water was made from a rock extract solution, in order to reproduce the chemical composition of infiltrating water that equilibrates with the host rock during percolation. A preliminary analysis of the influent water anions (except carbonates) indicated 26 ppm Cl^- , 46 ppm NO_3^- and 821 ppm SO_4^{2-} . Fluorescein was added to the water in concentrations ranging from 100 mg/L to 1 g/L until a final concentration of 200 mg/L proved to be the minimum concentration that allowed flow visualization. The water also contained 10 mg/L of glucose and 75 $\mu\text{g/L}$ each of fungicides cycloheximide and nyastatin. A syringe pump delivered the solution at a constant flow rate of 0.33 mL/hr. Strips of glass-fiber filter paper were placed along the top and bottom of the fracture for flow distribution and collection. The fracture was inclined at a sub-horizontal angle (30°). Effluent was collected below the fracture outlet and was periodically extracted by syringe for sample analysis (see later).

Open Geocosm

Figure 2.2 shows the seepage patterns observed on the two rock samples used in the open geocosm. They exhibited many of the features observed in the replica studies and theoretical modeling described in Geller et al. (1996), such as channelized flow and residual ponds connected by thin threads. However flow intermittence (the cyclic snapping and reforming of the channels) was not observed due to the absence of narrow apertures. In the outcrop sample, fungal growth spread over the rock surface several days after the test began. The appearance and tenacity of the fungi raises interesting questions regarding the general absence (or lack of observation) of fungal growth in vadose zone rock samples, despite their ubiquity in soil horizons. The fungi in the open geocosm most likely originated from air deposition onto the outcrop surface, as they did not appear on the core sample. Fungi are hydrophobic and any surface they cover becomes non-wetting to water. Due to the difficulty in eliminating fungal spores, further open geocosm tests were not conducted.

Rock-Replica Geocosm

The study of the rock-replica geocosm was motivated by the expectations that fungi would not grow in the apertures between the rock and replica due to inadequate open space and air flow, and that flow effects due to microbial activity would be greater in the presence of capillary forces induced by the aperture field compared to the open fracture surface. In this experiment, the rock and replica were initially sterilized and imprinted onto agar medium by cloth transfer to verify sterilization. The rock surface was inoculated with a

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mixed culture of micro-organisms derived from rock samples from TAN 33, INEEL obtained in 1996 (Geller et al., 1997). Colony forming units (CFU) were scraped from two agar plates and suspended in ten mL of a buffer solution and sprayed onto the fracture surface. This gave an estimated initial coverage of 7×10^7 organisms/cm², based on the number and size of CFU taken from the agar plates. The geocosm box and all associated tubing and fittings were sterilized before inserting the rock-replica assembly.

The flow channel was initially a single thread that extended the length of the fracture and periodically snapped near the bottom of the fracture plane, shown in Figure 2.3. After several days, a residual liquid pool formed in the channel path about one third the way down the fracture plane (Fig. 2.4). The presence of residual liquid pools in the path of the flow channel dramatically affected flow patterns, acting as reservoirs that underwent cycles of filling and partial drainage. The thread below the residual pools only formed as the up-gradient residual pool drained, and subsequently snapped. By this time, water also ponded near the fracture outlet, indicating that the filter paper used to collect the effluent had clogged, presumably due to bacterial growth.

Figure 2.5 (a) is a plot of the accumulated volume of water supplied to the geocosm (from the volume indicated on the syringe pump) and collected in the effluent. The water out is both the water collected as samples from the effluent collection, and water that leaked from the collection system as the effluent collection filter became clogged. The displacement of the two curves over the first two weeks of the experiment indicates the dead volume in the system. The same slope of the two curves shows that absorption of water by the rock matrix was not measurable. Once the leaks from the effluent collection began, the effluent arrangement was changed, which eliminated the leakage, but added dead-volume to the system.

The fracture effluent was analyzed for CFU by plating onto soil-rock extract agar, effluent surface tension, conductivity and pH and compared with the influent (Figure 2.5 (b)-(e)). Effluent CFU were on the order of 1×10^7 to 1×10^8 per mL, with a possible increasing trend during the experiment (Fig 2.5(b)). Countable plates (plates with 30 to 300 CFU) showed the presence of a mixed culture, including organisms of varied pigmentation and tendency to spread (Figs 2.6(a) and (b)). Plates crowded with more than 300 CFU did not exhibit varied pigmentation. Although the influent was filter-sterilized before application, it became contaminated and the cultures on the plates appeared mostly

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homogeneous (i.e. a single color) (Fig. 2.6(c)), suggesting that the contamination came from a single organism and not the mixed culture of the effluent. Identification of the cultured organisms is in progress, using fatty acid methylester (FAME) analysis. Preliminary identification of half of the 20 isolates from this experiment shows that these are the same micro-organisms identified in the core sample isolates from which the mixed culture was obtained.

To see if there was a correspondence between the distribution of CFU and water seepage patterns, the rock and replica were disassembled at the end of the experiment and agar imprints were made of the rock and replica surfaces by cloth transfer. The rock was imprinted both onto a soil-rock extract (SRE) agar with fungicides and onto an arginine glycerol salts (AGS) medium. The replica was imprinted only onto the SRE agar. Photographs of the agar plates five days after imprinting are shown in Figure 2.7.

On the AGS medium, the growth of organisms was confined to the area of the imprint, suggesting that there was no contamination during the imprinting procedure. Spreading organisms were observed on the AGS medium, which appear both within and outside the flow path of the seepage water. The range of pigmentation present in the plated effluent samples was not observed, probably due to the high concentration of organisms on the rock surface. In future experiments, lower initial surface concentrations of organisms should improve the ability to discriminate CFU within and outside the path of the seepage water.

On the SRE medium, the white areas along the edges of the imprinted areas of both the rock and replica indicate the presence of fungi, despite the addition of fungicides to the agar before pouring. This corresponds to the observation of fungal growth on the rock fracture surface that was not covered by the replica. There is no observable difference between the rock and replica imprints onto the SRE medium. The fact that fungi were not observed on the AGS medium suggests that the naturally occurring constituents in the SRE are conducive to fungal growth in the absence of free water.

Conclusions

The rock-replica geocosm experiment established the methodology for environmental controls and monitoring of future experiments. For the high initial concentration of microbes on the rock surface which are likely to be orders of magnitude greater than those reported at the site, water seepage patterns changed with time due to

2. Task 1. Phenomenological studies: open and rock-replica geocosms

bacterial activity. Future experiments are planned for lower initial surface concentrations of bacteria that are more representative of subsurface conditions, and to test the effect of the presence of a NAPL contaminant. The observations of the relationship between residual liquid pools and channel snapping should be incorporated into the development of the flow dynamics conceptual model.

Figure 2.1 Schematic description of environmental control chamber for rock-replica geocosm

(a) Open geocosm with natural fracture in dense basalt core

(b) Open geocosm with natural fracture from outcrop

Figure 2.2 Seepage in open geocosms. Left-hand figures are under room light, right-hand figures are under near-UV light.

Figure 2.3. Sequence of flow-channel formation and snapping in rock-replica geocosm.

Figure 2.4. Seepage in rock-replica geocosm in presence of residual water

Figure 2.5 Effluent measurements during rock-replica experiment

(a) Effluent, sampled 11/6/97 and 11/7/97

(b) Effluent, sampled 11/19/97 and 11/20/97

(c) Influent, sampled 11/14/97

Figure 2.6 Colony forming units on agar plates from rock-replica geocosm

(a) Imprint of rock on arginine-glycerol-salts medium

(b) Imprint of rock on soil/rock extract with fungicides medium

(c) Imprint of replica on soil/rock extract with fungicides medium

Figure 2.7 Imprint of rock and replica by cloth transfer after experiment

3. Task 2. Measurement of two- and three-phase flow in fractures

Overview

The approach in this task is to first directly observe liquid seepage behavior in transparent replicas of natural fractures, and then to study seepage behavior in a more quantitative manner as a function of boundary conditions, fracture inclination and liquid properties. Our observations of pervasive liquid channeling and snapping and reforming of these channels, and the importance this behavior has for quantifying liquid residence time and contaminant transport, motivated our work on characterizing intermittent flow behavior in terms of measurable parameters and dimensionless numbers. The fracture replicas allow the study of flow behavior as a function of fracture geometry, however their surface properties differ from the natural rock. As an intermediate step between the replica and natural rock, we are also conducting tests in a rock-replica combination where liquid distribution can be observed, but one half of the solid matrix is representative of natural minerals. The combination of flow visualization and tracer tests in transparent fracture replicas facilitates the interpretation of breakthrough curves for varying flow conditions that can then be applied in natural rock samples.

Characterization of intermittent flow

The cyclic snapping and reformation of flow channels (i.e. intermittent flow) occurs in all of the flow cells we have studied, including fracture replicas, rock-replica combinations and parallel glass plates of small-medium-small aperture sequences (shown in Table 3.1), as well as for different liquids. Both finger velocity and the frequency of intermittent flow events increase with increasing angle of inclination, and with increasing flow rate. We define Capillary (Ca) and Bond (Bo) Numbers in terms of finger velocity (V) and aperture at the point of channel snapping (b), respectively:

$$Ca = \frac{\mu V}{\sigma \cos \theta}$$
$$Bo = \frac{\rho b^2 g \sin \phi}{\sigma \cos \theta}$$

where μ is the liquid viscosity, σ is the air/liquid surface tension, θ is the contact angle of the liquid on the solid, measured through the liquid phase, ρ is the liquid density, g is the acceleration of gravity, and ϕ is the angle of inclination of the fracture from the horizontal.

3. Task 2. Measurement of two- and three-phase flow in fractures

Finger velocity is the rate of advance of the liquid meniscus following a channel snap, measured from digitized video images of the experiment.

Figure 3.1 plots the frequency of intermittent flow events, and the volume of liquid metered per flow event as a function of Ca and Bo . This analysis reveals consistent trends for water-seepage in the various flow cells. A manuscript for journal publication describing this work has been prepared, reviewed internally and submitted to Water Resources Research. This analysis will be extended to other liquids and flow cells studied in FY98.

Table 3.1: Water Seepage Experimental Conditions

Experiment	Flow cell	Loading	Inlet conditions	Q_m (ml/hr)	ϕ
A	epoxy fracture replica (FY97)	(1)	(3)	2.0	19°, 46°, 81.5°
B	epoxy fracture replica (FY96)	(2)	(4)	1.3 to 6.1	70°
C	glass parallel plates medium-small-large aperture sequence (FY976)	(1)	(5)	8.8 to 11.8 8.6 to 23.7	58° 70°
D	glass parallel plates small-large-medium aperture sequence (FY97)	(1)	(3)	2.0 to 5.0	76°

(1) Compressed between 1/2" lucite plates. (2) 35 kPa nitrogen confining pressure. (3) Ceramic endcap across middle third of inlet; water supplied by syringe pump. (4) Ceramic endcap across middle third of inlet; water supplied by Mariotte bottle. (5) Felt across middle third of inlet; water supplied by Mariotte bottle and flow rate controlled by a needle valve.

Seepage in a fracture replica as a function of liquid properties

Seepage of n-dodecane (less dense and more viscous than water), PCE (denser and less viscous than water) and water in an initially dry fracture at angles ranging from sub-horizontal to sub-vertical at a constant flow rate were compared. Table 3.2 lists the pertinent properties of these liquids. The frequency of intermittent flow events was much greater for NAPL seepage compared to water seepage, due to increased lateral spreading of the NAPLs compared to water (Figure 3.2). However the spatial liquid distributions of each liquid were similar, with residual liquid and flowing channels forming in the same location in each experiment (Figure 3.3). The role of liquid properties in small-large-small glass plate aperture sequences will be studied in 1998, as well as NAPL seepage into initially water-saturated replicas.

3. Task 2. Measurement of two- and three-phase flow in fractures

Table 3.2: Properties of liquids used in the seepage experiments

Liquid	Density (kg/m ³)	Viscosity (10 ⁻³ kg/(m-s))	Air/liquid surface tension (mN/m)	Contact angle on epoxy (degrees)	Vapor pressure (mm Hg)
Water (with 0.4% Liquitint)	995.6	1.103	64.4	62	17.6
n-dodecane	745	1.378	24.9	0	0.12
PCE	1630	0.89	31.7	0	18.47

Contact angles measured with goniometer; other properties obtained from literature, with citations available upon request.

Water seepage in a rock-replica combination

One half of the fracture replica used in the seepage experiments was mated with the rock sample of the second half of the fracture. Due to the size of the rock, a special holder was constructed to vary the angle of inclination of the fracture and bolt the replica to the rock (Figure 3.4). Seepage of water dyed with fluorescein was imaged under near-UV light. Figure 3.5 shows the invasion of water into the dry fracture at 5 mL/hr for a subhorizontal angle of inclination (31°). The frequency of intermittent flow events was about half of the value in the replica-only cell and the extent of liquid trapping was much smaller. This may be due to the different loading conditions, producing larger apertures in the rock-replica combination compared to the replica-replica, and wettability. This assembly will be used for more systematic study of flow behavior in FY98.

Tracer Tests in a Transparent Fracture Replica

The travel time of water through the fracture replica was measured with an electrolyte tracer with a step-function input. Gold wire electrodes were emplaced in the fracture inlet and outlet to measure the change in conductance of liquid with time (calibration verified that the solution conductance is linearly related to the tracer concentration). Tests were conducted at two flow rates, each at three angles of inclination (21°, 46°, and 81°). The breakthrough curves for 5 mL/hr at 81° are shown in Figure 3.6, for the liquid distribution shown in Figure 3.7. The fluctuations in the breakthrough curve are due to intermittent flow events. The mean travel times increase with increasing angle inclination (Table 3.3), which is contrary to both what we would expect as the relative strength of gravity increases and the measured increase in finger velocities at higher angles of inclination. The increase in travel times can be explained by the greater frequency of intermittent flow events at higher angles, which holds-up the solute longer because the time

3. Task 2. Measurement of two- and three-phase flow in fractures

the channel is disconnected increases. In FY98, similar tests will be used to characterize flow in rock-replica and rock-rock samples, and in three-phase flow conditions.

Table 3.3: Summary of average travel times and seepage velocities from tracer tests in the fracture replica				
Experiment	Q (ml/hr)	ϕ	average travel time (minutes)	average seepage velocity (cm/min) ^(c)
E	^(a) 5	21	15.0	2.13
		46	16.5	1.94
		81	19.0	1.68
F	^(b) 3	21	23.0	1.39
		46	30.5	1.05
		81	35.5	0.90

^(a) fracture raised from 21° to 81°

^(b) fracture angle decreased from 81° to 21°

^(c) travel distance is 32 cm, the length of the fracture

Figure 3.1: Volume of water metered between events as a function of (a) flow rate (b) Bond number and (c) Capillary number for Experiments A-D.

Figure 3.2. Frequency of intermittent flow events as a function of Bond Number

Figure 3.3. Liquid distribution during seepage of water, n-Dodecane and PCE into initially dry fracture replica.

Figure 3.4. Large rock-replica assembly

Figure 3.5 Initial invasion of water into the rock-replica. $Q = 5 \text{ mL/hr}$, 31 degrees

(a) fracture inlet

(b) fracture outlet

Figure 3.6. Breakthrough curves from tracer test for $Q_{in} = 5$ ml/hr, $\phi = 81$ degrees

Figure 3.7: Tracer test flow channel. $Q_{in} = 5$ ml/hr, 81 degrees

4. Task 3. Biotransformation of Volatile NAPL Organic Contaminants

Overview

The major research activities in 1997 in this task include (1) developing a protocol to prepare thin-sections of rock samples aseptically with minimum disturbance of the existing form and arrangement of microorganisms inside rock samples, and (2) developing a reflection Fourier transform infrared (FT-IR) spectromicroscopy technique to nondestructively monitor microbial population and their interactions with volatile NAPL organic contaminants. Rock samples were 3.25" diameter rock cores collected aseptically from the deep subsurface at INEEL (Geller et al., 1997). The construction of the geocosm models for these 3.25" rock cores did not begin until the end of FY97 due to the delay in the delivery of some key components for the model.

Thin Sectioning of Rock Samples

The goal of this effort was to provide a tool, prior to any major experimental effort, that could prepare thin-sections of rock sample aseptically with minimum disturbance of the existing form and arrangement of microorganisms inside the rock samples. The very nature (elevated temperature and large contact area between the rock sample and the saw blade) of rock-cutting often precludes the ability to maintain the integrity of the samples, making this a challenging task. We worked with the staff at the Applied Technology Ceramic Shop at the Lawrence Berkeley National Lab and developed a protocol for aseptic rock slicing. The protocol is a modified version of the existing low-speed diamond-wire-cutting technology used by the researchers in the area of advanced material sciences to investigate the structure, surface physics and surface chemistry of a crystal.

The low-speed saw is kept in a sterile laminar flow hood. Our protocol involves sterilizing the saw under UV irradiation twenty-four hours prior to its operation, and using filter-sterilized water during its operation. The efficacy of our protocol was confirmed by the following two observations. First, no colony-forming units appeared on an agar disc cut from a sterile agar sausage. The agar sausage was prepared by the technique described by Hirsch et al. (1995). Second, there was no detectable change of the form and arrangement of microorganisms on an agar disc cut from an agar sausage with known microbial distribution. This agar sausage was prepared by inoculating isolates from the vesicular basalt (Geller et al., 1997) into the agar sausage when the agar medium was still semisolid.

4. Biotransformation of Volatile NAPL Organic Contaminants

Stand-Alone and Synchrotron-Based IR Microspectroscopy Techniques

To monitor nondestructively the *in situ* response of the microbial population and activity to TCE and toluene on fractured rocks, and to establish the interaction mechanisms at the VOC-microorganism interface is a challenge. This is especially difficult when the concentrations of VOCs and microorganisms are at their environmental dilute levels. The mechanisms of transforming TCE and toluene may change during the microbial life cycle of attachment, growth, starvation, environmental stresses, and re-growth. The spatial heterogeneity of microbial communities and mineral compositions further complicate the problem. The "dilute" nature of both the lithotrophs and contaminants in the fractured-rock system, together with the changing cause-and-response process at the molecular-scale, has precluded our use of traditional macroscopic methods such as using ground minerals in batch or column reactors to yield measurements of biotransformation kinetics (e.g. Fuller et al., 1995). Traditional microscopic tools such as the scanning electron microscope (SEM) with energy-dispersive X-ray (EDX) microanalysis of samples that have received vacuum treatment only, documents the presence of microorganisms with respect to mineral patterns (e.g. Bouabid et al., 1995). Use of the newly developed atomic-force microscopy (AFM) techniques has answered the question of whether or not biomolecules interact with metal oxides (Maurice et al., 1996). To elucidate the biochemical interaction mechanisms at the VOC and bacterial interfaces in our fracture-rock systems, one needs to nondestructively examine and monitor the *in situ* structural changes of the VOC and biomolecules as the reactions take place.

A non-synchrotron-based Fourier transform infrared (FTIR) microspectroscopy combines the use of a microscope in conjunction with infrared spectral analysis (Reffner and Martoglio, 1995) to study structural changes of organic molecules as interactions take place in both biological and non-biological systems. The microscope provides a means to resolve microstructure detail and record the image for future data interpretation. Infrared spectroscopy provides a means for analyzing the chemistry of the sample at a molecular level in the infrared region of 400 cm^{-1} to 4000 cm^{-1} inverse wavelengths. This region is the molecular fingerprint region that exhibits unique vibrational features of many organic and biomolecules (Carr et al., 1995). The changes can even be followed quantitatively by using band intensity ratio techniques in conjunction with the selection of appropriate infrared reference standards (Lijour et al., 1994).

A review of the literature in the areas of biogeochemistry or geochemistry has yielded little information on the application of the non-synchrotron-based Fourier transform infrared microspectroscopy for characterizing a biogeochemical system. To

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achieve our project goal, we evaluated the potential of the infrared microspectroscopy technique in the area of biogeochemistry. The preliminary results were presented at the "Applied and Environmental Microbiology" (August 17-22, 1997) Gordon Conference. The abstract of a manuscript submitted for publication appears in the appendix of this report.

We used FTIR to monitor the spectral changes of lithotrophs' extracellular polymers as the microbes grew under changing growth conditions (relative humidity) during the experiment. All of the organically-derived biological molecules that are inherent to our biogeochemical system possess functional groups that can be monitored as components of molecular species. These functional groups can also be monitored as these same molecular species are perturbed by interactions with water and other constituents such as metal ions (in minerals).

The study consisted of conducting both reference and environmental experiments. The reference experiment was designed to provide us with spectral information relevant to our biogeochemical system. The experiment involved incubating isolates of intrinsic lithotrophs on an aluminized microscope slide that was coated with a thin film of basalt extract at room temperature. The aluminized microscope slide was for the purpose of surface enhancement to improve the signal-to-noise levels relative to our previous screening studies. The environmental experiments involved incubating a thin-section of the basalt sample under the same experimental conditions as the reference experiments but without the benefit of the surface enhancement.

Because of the presence of moisture, our infrared spectra were obtained using the method that has been published in the literature for taking high quality spectra successfully in both the transmission and reflectance modes in the presence of moisture (Reeves, 1995a,b). The assignment of infrared vibration bands for the microorganisms is based on Brandenburg and Seydel (1996). For the mineral bands, the assignments are based on the U.S. Geological Survey's Mineral Library. The shifting and the ratios of integrated areas of vibration bands were used to study the responses of microorganisms to the changing relative humidity.

Preliminary results indicated that the quality (i.e. signal-to-noise level) of spectra from the reference experiments was excellent. Figure 4.1 shows the FTIR spectra recorded from the Gram-positive and Gram-negative basalt-inhabiting bacteria. Spectral bands representing the organic functional groups that were part of our biogeochemical systems (including ester, carboxylic acid, ether, and hydroxy groups) were similar to those reported in the literature (Brandenburg and Seydel, 1996; Rammelsberg et al., 1997). The peaks at wavenumbers 3000 to 2800 cm^{-1} correspond to the functional groups

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of fatty acids of various membrane amphiphiles of the bacteria cell wall. Peaks at wavenumbers 1650 to 1550 cm^{-1} correspond to the Amide I and Amide II protein, respectively.

The quality of the spectra from the environmental experiments was less than ideal. The signal-to-noise level was lower than that observed in the reference experiment and spectral features could not be compared with those that have been observed in the reference experiments. However, the signal-to-noise ratio was sufficient to identify effects of moisture on the microbial growth on the rock surface, shown in Figure 4.2. Figure 4.2(a) shows the spectrum of the initial microbial distribution. The absorbance intensity of the biomolecules decreases significantly at the lower relative humidity (23%), shown in Figure 4.2(b). This decrease is due to changes in cell size and morphology, which was confirmed by direct observation under a microscope. Upon increasing the relative humidity to 100%, the absorbance intensity recovers, shown in Figure 4.2(c).

We believe that we can improve the signal-to-noise ratio by linking the analytical system to the National Synchrotron Infrared source whose brightness is between 100 to 1000 times higher than the conventional infrared source (Reffner and Martoglio, 1994). The additional high spatial resolution supplied by the synchrotron infrared source would enable us to monitor biochemical processes at a scale of one to several μm , only slightly larger than the size of a bacterium.

Instrumentation for isotope analysis

In conjunction with the purchase of a new stable isotope mass spectrometer at the Center for Isotope Geochemistry (CIG), a pre-concentration system was acquired for use with this project. The new mass spectrometer is a bench-top model designed for continuous flow applications. For conventional dual inlet machines (the type of system CIG currently has), only a small fraction of the sample is actually utilized. In the continuous flow method, the sample is introduced into the mass spectrometer in a stream of carrier gas and the isotope ratios are measured by integrating the signal collected for the entire sample. The result is that the isotope ratios of much smaller amounts of gas (10^{-2} to 10^{-3} the size) can be measured with nearly the same precision by the continuous flow instruments.

The pre-concentration accessory is an automated preparation system that separates gas samples with cryogenic traps and gas chromatographic columns before admitting the sample into the mass spectrometer. It is specifically designed for measuring compounds such as CO_2 and CH_4 in air samples, although it will be modified for measuring VOCs. This greatly enhances our ability to detect shifts in the isotopic

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compositions of gases that indicate microbial metabolic activity is occurring during our laboratory experiments. Another advantage of this accessory is that the increased sensitivity of the system allows us to use stable isotope labeled substrates for our experiments instead of compounds labeled with radiocarbon, minimizing the amount of mixed hazardous waste produced.

Fig. 4.1 The FTIR spectra recorded from the (a) Gram-positive and (b) Gram-negative basalt inhabiting bacteria on an aluminized microscope slide coated with a thin film of basalt extract. The relative humidity was changed from 100% to 23% and back to 100%.

Fig. 4.2 A series of spatial spectra obtained during the in situ experiments. (a) Initial microbial distribution, (b) in 23% RH for 15 days, and (3) in 100% RH for 10 days.

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5. Task 4. Numerical Simulation Experiments on Water Seepage in Heterogeneous Fractures

Introduction

For purposes of theoretical analysis, "small-aperture" fractures in hard rocks of low matrix permeability have been conceptualized as two-dimensional heterogeneous porous media (Pruess and Tsang, 1990). Multiphase flow in such fractures has been modeled with macroscale continuum concepts, such as a multiphase extension of Darcy's law that includes relative permeability and capillary pressure effects (Pruess, 1998).

They key to realistic modeling of multiphase flow in heterogeneous fractures is a realistic representation of the permeability structure in the fracture plane. Aspects of fracture aperture distribution that are believed to be essential for replicating natural features include (a) the presence of asperity contacts, where the fracture walls touch, (b) a more or less gradual change towards larger apertures away from the asperities, (c) small-scale fracture wall roughness, and (d) finite-size spatial correlation length among apertures (Wang and Narasimhan, 1985; Pruess and Antunez, 1995).

Simulated annealing (SA) is an optimization algorithm that draws an analogy between the thermodynamic processes of annealing and an optimal ordering, e.g., minimum energy, of a system with various components. Four elements are necessary in any SA algorithm: (1) an initial state, (2) an objective (energy) function, (3) perturbation mechanism and acceptance criteria, and (4) annealing schedule. For the purpose of characterizing heterogeneous fractures, for example, the component in SA could be the permeability field, and the objective function could be a measure of the spatial correlation structure of the system, e.g., the semi-variogram of the permeability field.

Spatially correlated heterogeneous permeability fields can be obtained by means of the turning bands method. However, this method is limited to log-normal distribution of "permeability modification coefficients." We have used "simulated annealing" (SA) as a more flexible approach for generating spatially correlated heterogeneous fields. SA can handle log-normal and non-Gaussian distributions. In this section we describe

5. Numerical Simulation Experiments on Water Seepage in Heterogeneous Fractures

modifications to the standard Metropolis algorithm that were necessitated by peculiar heterogeneity features of fractures (e.g., presence of asperity contacts), and we demonstrate applications of synthetic heterogeneous fields for modeling unsaturated flow of water.

Numerical experiments and results

Consider a 20m×20m vertical fracture plane discretized into 100×100 square grid blocks. The permeability at each grid block is defined as $k=k_{\text{ref}}\times\zeta$ where k_{ref} is a reference permeability and ζ is the permeability modifier. To characterize the heterogeneous permeability field, one can use SA to perturb the ζ field such that the optimal ζ field, or the permeability field, follows a specific correlation structure.

In all the following illustrations, the initial asperity contacts (10% of total sample data) are simulated as random, anisotropic ellipses and are used as conditioning data in SA, i.e., their values and positions are never perturbed. In addition to the conditioning asperity contacts, the initial ζ field is supplemented by drawing variates from a log-normal distribution with mean($\mu_{\ln\zeta}$)=1.0, and standard deviation($\sigma_{\ln\zeta}$)=1.5. In order to have a gradual change from asperities toward larger apertures, the initial ζ field is shifted by a constant Δ , i.e., $\zeta'=\max(0, \zeta-\Delta)$, such that the total percentage of asperity contacts increases from 10% to 25%. Then the initial random ζ' field is perturbed according to a particular perturbation mechanism until the system reaches the state with the minimum objective function. In our SA algorithm, a normalized and non-dimensional objective function is defined in terms of the squared difference between the semi-variograms of the desired distribution and the realization. The spatial correlation is assumed to be an isotropic exponential semi-variogram with nugget=0, sill=190, and integral scale=0.2m.

The perturbation mechanism used in our SA algorithm is simply swapping two randomly chosen ζ' values at two non-conditional grid blocks. To ensure that a global minimum objective function is reached, Metropolis et. al. (1953) proposed an algorithm in which a perturbation with decreasing objective function is always favored but a perturbation with increasing objective function can also be accepted with a certain

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probability. This acceptance probability is an exponential function of the parameter 'temperature' that will be decreased according to the annealing schedule.

However, our studies have shown that this standard Metropolis algorithm is not able to produce a permeability field with a smooth change of ζ' from asperity contacts to large ζ' . Therefore, the standard Metropolis algorithm was modified by introducing the concept of 'neighborhood'. Two different kinds of neighborhood are defined as shown in Figure 1, such that the first kind considers all grid blocks in a squared area around an asperity contact, while the second kind considers only grid blocks within a certain distance from an asperity contact. Thus, a perturbation is still favored if the objective function decreases. If, however, the objective function increases after a perturbation, the perturbation is rejected or accepted depending on whether or not the grid blocks are located in some neighborhoods. The modified Metropolis algorithm is explained in the flowchart shown in Figure 5.2.

Our studies show that annealing results depend strongly on the characteristics of the underlying probability distribution of the ζ field. For example, if a shifted log-normal and a step-wise uniform distribution are used (see Figure 5.3 for corresponding probability density functions(pdf's) and cumulative distribution functions(CDF's)), the annealing results shown in Figure 5.4 are quite different. First, for both distributions, annealing results from the modified Metropolis algorithm all have obvious clusters around the asperity contacts (red) than from standard Metropolis algorithm. Second, annealing results for a CDF curve having a smaller slope from $\zeta=0$ are more likely to have significant clustering effect around asperity contacts than those for a CDF curve having a larger slope from $\zeta=0$. This is because SA doesn't impose too much penalty on the pairs with two small ζ 's. In other words, the more large ζ a distribution has, the more likely obvious clusters will be formed around asperity contacts. If, however, $\mu_{\ln\zeta}$ is increased from 1.0 to 1.5, the clustering effect for annealing results from modified Metropolis algorithm is more significant in this case than the case with $\mu_{\ln\zeta}=1.0$; compare Figure 5.5 and Figure 5.4.

5. Numerical Simulation Experiments on Water Seepage in Heterogeneous Fractures

Permeability fields such as shown in Figures 5.4 and 5.5 were used to simulate water seepage by means of the TOUGH2 simulator (Pruess, 1991). Figure 5.6 shows the liquid seepage for water at breakthrough time in the heterogeneous fractures of Figures 5.4(a)-5.4(c) for a uniformly distributed infiltration over the top boundary at a constant rate of 10^{-3} kg/s. Water migrates downward from the top boundary in a non-uniform fashion due to the heterogeneity of the permeability field. At some depth below the top boundary, water begins to develop various fingers with different sizes. These fingers may proceed downward, merge with other fingers, pond upon asperity contacts, or be diverted by asperity contacts. The breakthrough points are all different for the three realizations shown in Figure 5.6. Furthermore, it is seen that seepage in Figures 5.6(b) and 5.6(c) has more ponded water than in Figure 5.6(a) because the permeability fields annealed with modified Metropolis algorithm have significant clusters. Figures 5.6(d)-5.6(f) show the realizations corresponding to Figures 5.6(a)-5.6(c) when the system reaches the steady state. It is seen that the fingers continue to evolve after the first breakthrough. The corresponding breakthrough curves are given in Figure 5.7; they show complex temporal structure.

Experimental data (Raven and Gale, 1985) have shown that when fractures undergo loading and unloading processes, e.g., by changing the normal stress, the discharges through fractures exhibit a hysteresis effect. Furthermore, as the normal stress increases, some openings in fractures may diminish while some asperity contacts may be propped open. Therefore, it is of interest to study the flow in fractures with different magnitude of normal stress. If shear stress is not considered and assuming that asperity contacts remain in contact with increasing normal stress, fractures under different magnitude of normal stress can be simulated as having different percentages of total asperity contacts. Using the same initial conditioning asperity contacts as in Figures 5.4 and 5.5, Figure 5.8 shows the permeability fields with different percentages (from 15% to 45%) of total asperity contacts obtained by changing the value of Δ . It is obvious from the corresponding seepage patterns shown in Figure 5.9 that fingers become thinner and

5. Numerical Simulation Experiments on Water Seepage in Heterogeneous Fractures

more tortuous, and ponding increases as the percentage of asperity contacts increases. Average effective permeability decreases with increasing percentage of asperity contacts, yet the breakthrough time may be shorter as seepage patterns become more sparse with increasing percentage of asperity contacts. In other words, unsaturated flow may be faster in less permeable fractures.

The numerical simulation experiments presented here are of a preliminary and exploratory nature. In future work we plan to use statistically meaningful ensembles of heterogeneous fields. In addition to variably-saturated flow of water and soil gas, we will also study three-phase flows of water, gas, and NAPLs.

(a) First kind of neighborhood with $M=3$

(b) Second kind of neighborhood with $M=3$

Figure 5.1 Schematic definitions of the neighborhood of an asperity contact. For an asperity contact, the neighborhood is defined as (a) the non-asperity grid blocks that are at most M blocks away from it, or (b) the non-asperity grid blocks with distances between the asperity contact and the grid blocks smaller than M . For example, the neighborhoods of the asperity contact located at $(0,0)$ with $M=3$ are the 48 and 28 gray blocks shown above for the first and second definitions, respectively.

Figure 5.2 Flowchart of the SA algorithm using modified Metropolis algorithm

Figure 5.3. Sample CDF's and pdf's for the two shifted log-normal distributions with different mean values and a step-wise uniform distribution. The log-normal pdf with mean=1.0 (top left-hand corner) is used in Figure 5.4, and the log-normal pdf with mean=1.5 below (second from top left-hand corner) is used in Figure 5.5.

Figure 5.4. Permeability fields annealed with standard and modified Metropolis algorithms for a log-normal distribution with $\mu_{\ln\zeta}=1.0$, $\sigma_{\ln\zeta}=1.5$, and $\Delta=0.64$ and a step-wise uniform distribution (see Figure 5.3 for corresponding pdf).

(a) standard Metropolis algorithm

(b) modified Metropolis algorithm, first kind of neighborhood

(c) modified Metropolis algorithm, second kind of neighborhood

Figure 5.5. Permeability fields annealed with standard and modified Metropolis algorithm using a shifted log-normal distribution with $\mu_{\ln\zeta}=1.0$, $\sigma_{\ln\zeta}=1.5$, and $\Delta=1.05$ (see Figure 5.3 for pdf).

Figure 5.6 Liquid saturation in heterogeneous fractures shown in fractures 5.4(a)-5.4(c) for first breakthrough and steady state

(a) Standard

(b) Modified, first neighborhood

(c) Modified, second neighborhood

Figure 5.7 Breakthrough curves of the flows shown in Figures 5.6(d)-5.6(f)

Figure 5.8 Permeability fields annealed with modified Metropolis algorithm for the first kind of neighborhood with different total percentage of asperity contacts.

Figure 5.9 Liquid saturation in heterogeneous fractures shown in Figure 5.8

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8. Appendix

8.1 Citations of publications generated from this project.

Pruess, K. On Water Seepage and Fast Preferential Flow in Heterogeneous, Unsaturated Rock Fractures, *J. Contam. Hydr.*, in press, 1998.

Pruess, K., B. Faybishenko, and G.S. Bodvarsson, Alternative Concepts and Approaches for Modeling Unsaturated Flow and Transport in Fractured Rocks, in: G.S. Bodvarsson (ed.), *The Site-Scale Unsaturated Zone Model of Yucca Mountain, Nevada, for Viability Assessment*, Lawrence Berkeley National Laboratory Report LBNL-40376, 1997.

8.2 Abstracts of papers submitted for publication and presented at conferences

H.-Y. N. Holman, D. L. Perry, and J. C. Hunter-Cevera, Surface-Enhanced Infrared Absorption-Reflectance (SEIRA) Microspectroscopy – a Chemical Probe for Investigating Bacteria Localization in Geologic Materials, submitted to the *Journal of Microbiology Methods*.

The applicability of surface-enhanced infrared absorption-reflectance (SEIRA) microspectroscopy as a chemical probe to provide insights into the localization of endolithic bacteria within geologic materials was investigated. Sample specimens were prepared from vesicular basalts collected aseptically at 65-70 m below the land surface in a subsurface rock vadose zone in southeastern Idaho. The surface-enhancement was achieved by evaporating a very thin gold-film on the specimen surface. Microspectra of the specimen surface were recorded in the $650\text{-}4000\text{ cm}^{-1}$ infrared region at a resolution of 4 cm^{-1} on a Fourier-transform infrared spectrometer coupled to an infrared microscope. Examination of the microspectra revealed that all bacteria-inhabiting surfaces exhibited very similar spectral characteristics. In particular, the sensitive absorption bands of carboxylic acid groups and the absorption envelopes of proteins amide I and II were found to be constantly centered around 1741 , 1651 and 1550 cm^{-1} , which became an ideal marker by which to detect the presence of bacteria. The reflectance intensity for basalt silicates in the $1300\text{-}800\text{ cm}^{-1}$ region was found to diminish in the presence of vesicles (gas-bubble cavities), which became a marker to detect the locations of vesicles. Comparative analysis of the space-resolved microspectra measurements suggested that bacteria in vadose vesicular basalts not only lived on the vesicle surface but also penetrated and lived inside spaces between clusters of larger vesicles. With this effort, the practical aspects and the usefulness of SEIRA as a promisingly simple and nondestructive analytical tool for studying the *in situ* localization of bacteria within rocks has been demonstrated.

8. Appendix

Pruess, K. A Mechanistic Model for Fast Preferential Flow in Unsaturated Fractured Rock.
Invited paper, presented at AGU Fall Meeting, San Francisco, CA, December 1997.

Observations at several field sites in semi-arid regions have shown that water can seep through thick unsaturated zones in fractured rock with pore velocities of the order of 10 m/year. This exceeds possible velocities of volume-averaged flow through the rock matrix by several orders of magnitude, providing clear evidence that some fraction of net infiltration occurs via localized preferential flow paths through the fracture network. Different conceptual approaches can be used to tackle the problem of water seepage in partially-saturated fractured rock. Current models of the Yucca Mountain site usually employ coarse space resolution and large-scale volume averaging over scales of tens to hundreds of meters. However, if an important component of flow is localized in preferential pathways, then much of the flow system volume may not participate. Under these circumstances a mechanistic description of flow based on volume-averaged concepts may not be possible.

Here we take the view that macroscale continuum concepts, such as relative permeability and capillary pressure, can be used to describe liquid seepage in partially saturated fractured rock, provided they are applied at the scale of the flow phenomena. In other words, water saturation, matric potential, and hydraulic conductivity fields in rock fractures must be described and resolved on the scale where water seepage is actually present. We employ Richards' equation to study the behavior of localized seeps in partially saturated rock fractures, using simple analytical models as well as detailed high-resolution numerical simulation. It is shown that different styles of medium heterogeneities can either disperse localized flows or, conversely, can funnel and focus spatially-distributed flows into localized paths.

8. Appendix

Su, G., J. T. Geller, K. Pruess and F. Wen, Experimental studies of water seepage and intermittent flow in unsaturated, rough-walled fractures, submitted to *Water Resources Research*.

Flow visualization experiments are conducted on a transparent replica of a natural, rough-walled rock fracture from the Stripa Mine, Sweden, for inlet conditions of constant pressure and flow rate over a range of angles of inclination. The experiments demonstrate that infiltrating water proceeds through unsaturated rock fractures along non-uniform, localized preferential flow paths. These localized flow channels diminish rock matrix-fracture interaction, accelerating groundwater travel in unsaturated fractured-porous media compared to conventional conceptual models based on volume averaging. Pervasive unsteady or intermittent flow is also observed in these experiments, where portions of flow channels snap and reform. A mechanism for intermittent flow is proposed and investigated in experiments conducted on parallel plates with an aperture sequence progressing from a small to large to small aperture. This geometry reproduces the intermittent flow behavior observed in the fracture replica. Quantitative measurements of the frequency of intermittent flow events and volume of water metered per event are obtained from the fracture replica and parallel plate experiments and compared using the Bond and Capillary Numbers. The volume of water metered per event provides more consistent results between the experiments, indicating that this is an important parameter for characterizing intermittent flow.