Fractosphere, the Site of Hydrogeological-microbial Interaction: Current and Future Perspectives

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Abstract

Beneath the base of gas-hydrate stability (BGHS), gas-hydrate is disintegrated into gas and water that form an "unsaturated zone". Some gas-hydrate belts are located in the plate convergent margins, where fluid flows in the sub-BGHS unsaturated zones may have hydrogeological relevance. The fluid flow in unsaturated zone has a complicated mechanism due to flows in both gas and liquid phases, and hydraulic interaction (exchange of water) between the fracture and matrix. Furthermore, the formation of biofilms on fracture surfaces possibly alters the hydraulic properties of fractured rock masses. For accurate evaluation of macroscopic hydraulic properties of an unsaturated fractured rock system, a laboratory experiment was performed using two rock blocks with a single-fracture in-between. Absorption of a part of the water flowing through the fracture was experimentally measured and quantitatively evaluated by a single parameter, "sorptivity". Some microorganisms were cultured in the laboratory to form biofilms on the rock surface and some colonized in inter-grain micro-cracks. Based on theoretical considerations, we also hypothesize the potential for biofilm-forming microorganisms to alter macroscopic hydraulic properties of unsaturated fractured rock masses.

Key words: fractured rock, unsaturated zone, hydraulic property, fracture-matrix interaction, biofilm

I. Introduction

Beneath the base of gas-hydrate stability (BGHS), gas-hydrate is disintegrated into gas and water due to high temperature. In this sub-seafloor free gas zone, pore space is occupied with both liquid and gas phases, and an "unsaturated zone" is formed. Some gas-hydrate belts are located in the plate convergent margins such as Nankai Trough, where freshwater possibly from smectite-to-illtite transition may migrate into the sub-BGHS unsaturated zones (Brown et al., 2001). Fluid flow in the unsaturated zone is a complicated mechanism due to flows in gas and liquid phases. Entry of gas into sediment/rock pores is realistic only when the capillary pressure exceeds a value commensurate with the radius of pore throats. This in turn provides a barrier to exsolution of gas bubbles from the
pore fluid. A pore radius greater than 25 microns would allow gas exsolution from a supersaturated capillary of 10 MPa, whereas effective pore radii in fine sediments are on the order of 0.1 to 1 micron (Clennell et al., 2000).

At the ODP Site 808 in Nankai Trough, Japan, it was reported that much freshwater is provided by dehydration of smectite (Brown et al., 2001). The sub-seafloor unsaturated zones of the Nankai Trough gas-hydrate belts are likely to be fractured as inferred from the occurrence of mega- to micro-faults (Park et al., 2002). Fluid flow in unsaturated fractured rock has not been studied in detail until recently. An earthquake may be induced by fluid flows through pre-existing fractures or may induce new fractures (Moore and Vrolijk, 1992). There may be "flushes" of overpressurized fluids (Schedl, 1992), which may dissolve and/or precipitate minerals on the fracture surfaces to form ore de novo (Wilkinson and Johnston, 1996).

When water is supplied to unsaturated fractured rock masses, water first flows through the fractures and a part of it is absorbed by the matrix. This further complicates the water migration mechanism. For fractured sedimentary rock, it is important to understand the hydraulic interactions (i.e., the exchange of water) between the fracture and matrix for an accurate evaluation of macroscopic hydraulic properties of unsaturated fractured rock masses.

It is also known that some microorganism species exist in such fracture habitats. Pedersen (1996) observed "light" δ¹³C ratio of calcite (CaCO₃) precipitated on the fracture surfaces, which implies the influence of microbiological activities. Observation of the fracture surfaces by SEM and EDX revealed the occurrence of carbon-rich particles that was most likely due to microbial colonization. Some laboratory experiments using a tube or glass plates confirmed that microorganisms form film-like colonies, referred to as "biofilms", on the surfaces (Pedersen, et al., 1996; Fletcher and Murphy, 2001). Lehman, et al. (2001) reported that biofilms were formed on actual rock fracture surfaces in the laboratory. Hydrophobic biofilms in a fracture of micro-meter width may make the fracture resistant to absorption. On the other hand, a hydrophilic biofilm may facilitate fluid flow through the fractures. Thus, the formation of biofilm possibly alters the hydraulic properties of fractured rock masses.

In this paper, a theoretical consideration of the water flow mechanism in fractured rock will be presented first including the interaction between fracture and rock matrix. Then, we report results from an experiment where the water content of rock block was measured to monitor absorption of water from an adjacent fracture through which water was absorbed. For the measurement of water content in rock, Time Domain Reflectometry (TDR), which is relatively new to rocks, was used based on the findings of Sakaki et al. (1998). Finally, we will present results of some laboratory experiments on biofilm-forming microorganisms, and discuss their potential effects on the alteration of macroscopic hydraulic properties of unsaturated fractured rock masses.

II. Moisture Migration in Unsaturated Porous Media

1) Governing equation for water flow in fracture

It is assumed that fractures in unsaturated rock masses are water-saturated when water
flow occurs. For a water-saturated fracture with a uniform aperture $2b$, the flow rate $q_f$ can be described by the following equation.

$$q_f = -\frac{\rho_w g (2b)^3}{12 \mu_w} J$$  \hspace{1cm} (1)

where, $\rho_w$ is the mass of water per unit volume ($=1.0 \text{ kg/m}^3$), $g$ is the gravity ($= 9.8 \text{ m/sec}^2$), $\mu_w$ is the viscosity of water ($= 1 \times 10^{-3} \text{ Ns/m}$), $J$ is the hydraulic gradient.

Eq. (1) implies that the flow rate in the fracture is proportional to $(2b)^3$, which is well-known as the “cubic law” (Witherspoon et al., 1980). If the aperture is halved the flow rate in the fracture is reduced by a factor of eight. This extreme sensitivity implies that changes in the aperture (e.g., due to formation of biofilms on the fracture surface) could alter the hydraulic properties of the rock masses significantly.

2) Governing equations for fracture-matrix interaction

One dimensional absorption of water into unsaturated porous medium can be expressed by the following equation known as the “non-linear diffusion equation” equivalent to the “Richards’ equation” (Charbeneau, 2000). The standard form of the Richards’ equation includes the gravity term, which does not appear in eq. (2). The reasons will be discussed in the later section.

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x} \left(D(\theta) \frac{\partial \theta}{\partial x} \right)$$  \hspace{1cm} (2)

where, $\theta$ is the volumetric water content, $t$ is the time, $D(\theta)$ is the hydraulic diffusivity, $x$ is the spatial coordinate. The hydraulic diffusivity $D(\theta)$ changes with water content and thus eq. (2) becomes non-linear. In soil physics, eq. (2) has been utilized to describe absorption of water into unsaturated soil. We will apply eq. (2) to quantify water absorption from fracture surface.

As a simple example, consider the following conditions. Initial water content before the absorption occurs occurs ($\theta_i$) is uniform. At the water-supply (fracture) surface $x = 0$ the absorption begins at time $t = 0$ and water content at $x = 0$ becomes $\theta = \theta_o$ instantaneously.

$$\theta = \theta_i, \text{ at } t = 0, \ x > 0$$

$$\theta = \theta_o \text{ at } t > 0, \ x = 0$$

Philip (1969) solved eq. (2) under above conditions and obtained the following analytical solution.

$$I(t) = S \sqrt{t}$$  \hspace{1cm} (3)

where, $I(t)$ is the cumulative absorption per unit area, $S$ is the sorptivity. Differentiating eq. (3) with respect to time yields,

$$i(t) = \frac{1}{2} S \cdot t^{-\frac{1}{2}}$$  \hspace{1cm} (4)

where, $i(t)$ is the instantaneous absorption rate per unit area. Eqs. (3) and (4) are known as the "Philip's equations". From eq. (3), the cumulative absorption per unit area of matrix is linearly proportional to the square root of time. Eq. (4) implies that the absorption rate becomes infinity at $t = 0$. However, in reality, quantity of water supply is limited and initial absorption rate is equal to the amount of external supply. When $i(t)$ becomes smaller than the supply rate, eq. (4) becomes valid. Consider an example of a ground surface when a precipitation event occurs. During the initial stage, most of water is absorbed into the ground and at a certain time it starts to
accumulate on the surface. This is because of
the decrease in $i(t)$ with time and also because
the external supply exceeds $i(t)$. For the
single-fracture wetting experiment to be
shown in this paper, the water supply rate
was determined such that the supply rate
exceeds $i(t)$ and eq. (4) becomes valid within
a few minutes after the experiment is
initiated.

3) Potential effect of microbial coloni-
zation and activity

It is known that some microbial species
exist in fractured rock environments and
possibly form film-like colonies, referred to as
"biofilms", on the surfaces (Pedersen, et al.,
1996; Fletcher and Murphy, 2001). Both the
degree of water saturation and the velocity of
the water may also influence bacterial trans-
port and biofilm formation. The importance of
water saturation can be seen in the unsatu-
rated zone where retention of water in the
pore spaces controls the transport of water.
As the water content of the unsaturated zone
decreases, water is contained in increasingly
small capillary spaces between the sediment
grains. Accordingly, as the thickness of the
water film in the fracture decreases, inhibi-
tion of biofilm formation on fracture surfaces
becomes significant. Reducing the distance
between the bacteria and the fracture surface
increases the probability that the bacteria
will contact the surface and form a biofilm
thereon.

Previous studies have shown that cell surface
hydrophobicity is a major factor controlling
the amount of bacterial colonization on
the fracture surfaces (Fletcher and Murphy,
2001). Microbial attachment to surfaces is
affected by physicochemical attractive/repul-
sive interactions and microbial adhesion
mechanisms. Once microorganisms colonize
and form biofilms on surfaces, a thicker
biofilms may serve as moisture retainers as
well as strainers/filters for gas bubbles,
colloids and particles. More importantly,
formation of such biofilms could change the
fracture aperture and alter the transmissivity
of the fracture significantly as inferred from
eq. (1).

III. Materials and Methods

Laboratory experiments for water migra-
tion and biofilm formation were conducted at
different locations using different rock sam-
pies. For the water migration mechanism, the
rock sample used for the laboratory experi-
ments was Indiana limestone from Indiana,
USA. Its effective porosity is 12–17% and its
saturated hydraulic conductivity is $1.5 \times 10^{-6}$
cm/sec. For the biofilm formation studies,
Kimachi sandstone, Shimane Pref., Japan
with an effective porosity of 21–22% was
used. These rocks were chosen because they
were uniform, isotropic, easy to obtain and
cut, and moderately porous.

1) Dielectric properties of Indiana lime-
stone

The dielectric constant is the index of how
the material resists electromagnetic waves. A
large dielectric constant means that the
transmission velocity of an electromagnetic
wave through the material is slow. Unsatu-
rated rock consists of solid (rock minerals),
liquid (water), and gas (air) phases. Dielectric
constants of rock minerals range from 3.8–7.5
(Shen et al., 1985) for most rocks and 7.5–9.2
for limestone (Wharton et al., 1980). Air and
water have dielectric constants of 1 and 81 (at
15 °C, 300 MHz: von Hippel, 1995), respec-
tively. Since the dielectric constant of water
is substantially larger than the other two, the
apparent dielectric constant of rock as a 3-
phase composite material strongly depends on water content. In the TDR method, an electrode is installed into the rock and the electromagnetic wave transmission velocity \( v \) along the electrode is measured. Then the dielectric constant of rock \( K_a \) is obtained from \( \varepsilon_\infty = \frac{c}{\sqrt{K_a}} \), where \( c = \text{speed of light} (= 3 \times 10^8 \text{ m/sec}) \).

To convert the dielectric constant into volumetric water content, a relationship between dielectric constant \( K_a \) and water content \( \theta \) is needed (hereafter, referred to as the \( K_a-\theta \) relationship). Thus, a calibration was conducted using small blocks of Indiana limestone. The dimensions of the blocks were \( 6 \times 6 \times 10 \text{ cm} \). A pair of aluminum tape strips with length \( l = 80 \text{ mm} \), width \( d = 3 \text{ mm} \), and spacing \( s = 8 \text{ mm} \) were attached onto the surface of the blocks. The blocks were placed in water in a vacuum chamber and left for more than 24 hours. Assuming that the blocks were saturated (Lin et al., 2000), the dielectric constant of the blocks was measured. Then, the blocks were air-dried. When the pre-determined masses were achieved, the blocks were wrapped and placed in a closed container for more than 24 hours for the water in the blocks to re-distribute and water content within the blocks to become uniform. Starting from 100% saturation to 0%, the measurements were taken at eleven water content levels (10% saturation increments). To achieve 0% saturation, the blocks were placed in an oven at 103\( ^\circ \text{C} \) for more than a week.

2) **Non-fractured matrix wetting experiment**

An Indiana limestone block with 37.7 (W) \( \times 28.4 \text{ (H)} \times 5.8 \text{ cm (T)} \) was completely dried in the oven at 103\( ^\circ \text{C} \) for over 48 hours. At the center of the top surface, a line source for water supply was set (Fig. 1). De-aired water was supplied by a pump continuously so that there was always a layer of water with a height of 1–2 mm. Excess water returned to the reservoir. By not pressurizing the water at the supply boundary, the pressure head did not affect the water absorption at the boundary. In this experiment, we observed how the wetting front migrates within the block with time.

3) **Single-fracture wetting experiment**

A schematic view of the experiment is shown in Fig. 2. Dimensions of the blocks were 16.8 (W) \( \times 26.8 \text{ (H)} \times 5.3 \text{ cm (T)} \). The blocks were dried in the oven at 103\( ^\circ \text{C} \) for more than 48 hours. The blocks were mated together so that a fracture is formed in the middle. They were placed on a metal frame so that the fracture was vertical. A moderate force was applied to push the blocks close to each other. The surface of the fracture was smooth and a 0.1 mm film was used as a spacer to ensure a uniform aperture. During the experiment, the blocks were covered by acrylic plates. It was expected that the wetting front would migrate horizontally from...
the fracture, therefore eight TDR probes were aligned such that water content distribution in the horizontal direction could be measured sufficiently. The probes were made of aluminum foil tape and a pair of the strips forms a probe. The dimensions of the strips were: length \( l = 100 \text{ mm} \), width \( d = 3 \text{ mm} \), spacing \( s = 8 \text{ mm} \). With these dimensions, water content up to a depth of 8 mm from the block surfaces could be measured. De-aired water was supplied by a pump at the top of the fracture and the flow rate was maintained at 2.2 cm\(^3\)/min (see section II.2). The bottom of the fracture was exposed to atmospheric pressure and outflowing water was collected in a container.

In this experiment, masses of the inflow and outflow reservoirs were measured. From the difference of these, the amount of water that was actually absorbed by the blocks was calculated. Water absorbed by the blocks was also estimated from the TDR measurements and these were compared to each other.

4) Biofilm formation experiment

Several pure cultures of microorganisms with various levels of cell surface hydrophobicity were obtained from various environmental samples including rocks. An unidentified bacterial species of the genus *Halomonas* that showed a very low hydrophobicity, *i.e.*, high hydrophilicity, was used for experimental biofilm formation on a fracture surface of Kimachi sandstone from Shimane Prefecture, Japan.

Pieces of Kimachi sandstone were crushed with a hammer, and the relatively flat fragments of 5–10 mm across were collected and sterilized by autoclaving for subsequent microbial culture experiments. Autoclaved sandstone fragments were immersed in a fresh microbiological medium (0.5% peptone, 0.25% yeast extract, 0.1% glucose and 4% NaOH in distilled water; pH ca. 7) at different salinities of 0.5% NaCl and 20% NaCl. This fresh medium with rock fragments was then added with portions of the pre-incubated *Halomonas* liquid culture and incubated at room temperature (ca. 20°C). After 3–7 days of incubation, the sandstone fragments were transferred to 50% ethanol to fix bacterial cells for SEM microscopy. After 30 minutes of 50% ethanol immersion, the rock fragments were then transferred to an ethanol-series 70%, 80%, 90%, 95%, 98%, 99%, and 100% ethanol for 30 minutes each immersion, which resulted in gentle and complete dehydration of bacterial cells for SEM observation.

IV. Results and Discussion

1) Dielectric properties of Indiana limestone

The dielectric constant values measured by aluminum foil tape probes at eleven water content levels are shown in Fig. 3.

The measured data were fitted with a second-order polynomial. The obtained function was;
For Indiana limestone, changes in dielectric constant due to changes in water content were large enough for accurate water content measurement. The dependence of $K_{\theta}$ on $\theta$ can be seen for most rocks but TDR may not be able to detect such changes for rocks with effective porosity of less than 1% (Schneebeli et al., 1995).

2) Non-fracture matrix wetting experiment

Figure 4 shows the wetting front migration at $t = 72$ hours. The shape of the front is semicircular around the water-supply boundary at the center of the top surface. The block was completely dry at $t = 0$ and water migration occurs mainly due to the matrix suction. This implies that for a relatively dry matrix, the effect of gravity becomes negligible. This is the reason why the gravity term in eq. (2) was omitted. Cases where the initial water content is not very low should be discussed in the future and are beyond the scope of this paper.

3) Single-fracture wetting experiment (water absorbed)

Cumulative absorption per unit area $I(t)$ is plotted against square root of time in Fig. 5. The gravimetric method, i.e., $I(t)$ calculated from the inflow and outflow reservoir masses is denoted as I-O. At $t = 60$ hours (thus, $\sqrt{t} = 7.7$ hours$^{1/2}$), the wetting front reached the other side of the block at $x = 16.8$ cm, which is directly related to the block size. Until a little after the front reached the other boundary, $I(t)$ was linearly related to $t^{1/2}$. It implies that the Philip's equations apply to the water absorption from fracture until the block size effect becomes significant. From the slope of the graph, sorptivity was calculated as $S =$

\[ \theta = -0.00103K_{\theta}^2 + 0.0438K_{\theta} - 0.220 \]  

\[ \text{(5)} \]
0.026 cm/min$^{1/2}$. Water content measurement by the dielectric method agreed well with the I-O method.

In Fig. 6, change in water content distribution in the right block is presented. These distributions were computed from the $K_\theta$ distribution in the block using the $K_\theta - \theta$ relationship (eq. (5)). The Y-axis ($x = 0$ cm) corresponds to the location of the fracture. It can be easily seen that the front reached the block boundary at $t = 60$ hours. The water content at the fracture surface ($x = 0$ cm) was not measured. However, the data within the block (until $t = 60$ hours at which the wetting front reached the other side of the block) suggest that the water content at $x = 0$ would have been approximately 0.09–0.12. Philip's equations assume that the water content at the water-supply boundary changes instantaneously at $t = 0$ and remains unchanged for $t > 0$ as described in Section II.2. In this experiment, the flow rate through the fracture was fixed to 2.2 cm$^3$/min. It does not ensure that the absorption rate or water content at the fracture surface is fixed. Nonetheless, the constant flow rate through the fracture resulted in a water content in the range of 0.09–0.12, at $x = 0$. These values seemed reasonably constant, indicating good agreement with Philip's equations.

In Fig. 7, wetting front migration at $t = 24$ hours is presented. The front was parallel to the vertical fracture in the middle of the block and migrated in the horizontal direction, showing little effect of gravity. The first five probes were in the wet region, thus the visual location of the absorption front is consistent with the water content distribution in Fig. 6.

The above results showed that due to the large contrast in the hydraulic conductivities of the matrix and fracture, water primarily flows through the fracture. However, especially when the matrix has low water content, absorption of water from the fracture surface into the matrix can affect the macroscopic hydraulic properties of rock masses. For a dry matrix, such absorption of water into the matrix followed Philip's equations until the effect of the block size became significant. A single parameter, "sorptivity" can be used to quantify absorption until the block size or other boundary effects become significant.
4) Single-fracture wetting experiment (water not absorbed)

In the previous section, water that had been absorbed by the matrix was discussed. In this section, water that had not been absorbed (outflow at the bottom of the fracture) is discussed. In Fig. 8, the ratio of outflow to inflow is plotted against time. According to the gravimetric method (I-O), at $t = 0.5$ hours, 56% of inflow came out of the system without being absorbed. Thus, 44% of supplied water had been absorbed by the matrix. At $t = 2, 6, 12$ hours, the outflow increased to 80%, 89%, and 94%, respectively. After $t = 24$ hours, consistently more than 97% of water just flowed through the system. During the initial stage, a part of the water flowing in the fracture was absorbed by the matrix, which would be significant enough to affect the hydraulic behavior of the rock masses. After a certain time, the absorption rate decreased with time and most of water that entered the system flowed out of it.

In a real rock mass, a number of fractures exist and if we consider the system used here as one of the many fractures, then water that flows out of the system goes into the next one.

It is not simple to estimate how long it will take for the fracture-matrix interaction to affect the macroscopic hydraulic behavior. It is probably related to rock type, fracture density (i.e., block size), aperture, initial water content, sorptivity and so on. Therefore, the numbers that were shown in this paper are specific to the system that we used here. To obtain a more general understanding, it is necessary to collect more data using various rock types, boundary conditions, and realistic fracture network geometries.

5) Biofilm formation experiment

Certain species of bacteria have the ability to vary or retain their cell surface hydrophobicity according to surrounding conditions. For example, as in Fig. 9, the commonly known gamma-Proteobacterial enterobacterium Escherichida coli exhibits a wide range of cell surface hydrophobicity according to the growth conditions such as salinity, while the euryhaline halophilic bacterium Halomonas sp. shows relatively constant hydrophobicity over a salinity range. Therefore, types of biofilm-forming bacteria may greatly influ-
ence the hydrophobic properties of the rock fracture surfaces. That is, at the salinity of 7% NaCl, whether the biofilm in question is formed by *E. coli* or *Halomonas* sp. will strongly influence the fracture surface hydrophobicity, and thus the properties of fluid flow and transport along the fracture will be affected accordingly.

Observation by electron scanning microscopy (SEM) revealed that the *Halomonas* cells attached and grew on surfaces of the mineral grains in this sandstone. No significant difference was observed between the 0.5% and 20% NaCl incubations. As the experimental bacterium, *Halomonas* sp., is known to maintain relatively a high hydrophilicity over a wide range of salinity, the experimental salinities of 0.5% and 20% NaCl may have caused only limited difference in hydrophobic/hydrophilic features of the cells on grain surfaces.

The *Halomonas* cells on smooth flat surfaces were easily observed (Fig. 10), while the cell growth within inter-grain cavities were difficult to identify. Aggregates (micro-colonies) of the attached cells on micro-cracks (grain boundary cracks) were also observed, which corresponds to the initial process of biofilm formation (Fig. 10). Micro-colonies resulted from *in situ* and *de novo* proliferation of attached cells, rather than the attachment of already proliferated cell aggregates. Therefore the occurrence of micro-colonies strongly suggests that the *Halomonas* cells proliferated *in situ* and *de novo* on grain surfaces.

Grain surfaces are known to adsorb charged substances such as organic acids and phosphate, which would serve as the nutrition for the attached microbial cells. The nutrition flux is controlled by the fracture flows. Some of the proliferated cells may be detached and emigrate to settle on new surfaces. The microbial transport is also controlled by the fracture flows. Theoretical and/or experimental models for explaining the interaction between microbial proliferation-transport and fracture flow are still in their infancy. However, we wish to emphasize the impor-
tance of this aspect in the hydrogeo-microbiological context.

Biofilms were also formed on wider areas of micro-cracks. The biofilm consisted of loosely interconnected cells, with the cells serving as the initiation cores of biofilm formation and as the pit-fillers of inter-grain cavities. Against the expectation of sheet-like or film-like structure, we have observed non sheet-like bacterial interconnections as the biofilm in most samples. It was thus difficult to determine the hydrophobicity of film-like biofilms by conventional methods such as the measurement of contact angle. Further technical developments are needed to establish the practical approaches to 1) measurement of attached cell surface hydrophobicity (free-living cell surface hydrophobicity is easily measurable), and 2) formation of real film-like biofilms of various hydrophobic features on various rock surfaces.

The degree of saturation varies over time depending on the rate and duration of the external water supply. It induces saturated-unsaturated cycles in rock masses, which are extreme environments for microorganisms. Furthermore, the conditions such as unsaturated zones containing sub-seafloor methane free gas, and salinity variation due to the inter-exchange between saline water (sea water extracted due to consolidation) and fresh water due to the smectite-illite transformation will be an even more extreme environment for microorganisms. The *Halomonas* species are known to adapt and proliferate over a wide range of salinity variation. Some *Halomonas*-like species are known to grow in anoxic conditions, and would possibly thrive in the subsurface salinity-variable unsaturated regimes. Although film-like biofilm was not observed in the experiments with the cultured *Halomonas* sp., loosely interconnected cells formed micro-colonies in micro-cracks. This itself may alter the hydraulic properties of the rock matrix. More importantly, it showed a possibility that microorganisms could form biofilms on fracture surfaces and reduce the pore throat apertures. This will significantly alter the transmissivity of fracture and thus the macroscopic behavior of fractured rock mass.

Biofilm formation on fracture surfaces involves a number of complicated processes such as (1) approach of microbial cells to the surfaces by passive transportation and/or active migration, (2) physicochemical attraction/repulsion, (3) adhesion by extracellular materials, (4) cell proliferation on the surfaces, and (5) cell proliferation forming detached daughter cells for the next colonization (Fletcher and Murphy, 2001). It should be noted that only part of the above-stated aspects were dealt with in this paper. Much remains for further investigation, to better understand the tangled phenomenon of biofilm formation.

**V. Conclusion**

To investigate the gas-liquid flow mechanisms in the sub-seafloor free gas zone, we performed a series of fundamental experiments. Using a rock block with and without a fracture, absorption of water into the matrix was experimentally measured and hydraulic interaction between the matrix and fracture of the rock was quantitatively evaluated. Then, we presented some experimental results and discussed the potential effect of biofilm-forming microorganisms on the alteration of macroscopic hydraulic properties of unsaturated fractured rock masses.

For the non-fracture matrix wetting experi-
ment, the shape of the front was semi-circular around the water-supply boundary. The block was completely dry at $t = 0$ and the effect of gravity turned out to be negligible. In the single-fracture wetting experiment, the cumulative absorption per unit area $I(t)$ was linearly related to $t^{1/2}$, implying that the Philip's equations apply to the water absorption from fracture and the behavior may be estimated by a single parameter, “sorptivity”. The sorptivity was calculated as $S = 0.026 \text{ cm/min}^{1/2}$. Changes in water content distribution showed the migration of the wetting front very well. The wetting front was parallel to the vertical fracture in the middle of the block and migrated in the horizontal direction showing little effect of gravity. When the matrix has low water content, water absorption from the fracture surface into the matrix can affect the macroscopic hydraulic properties of the rock masses. The fact that the effect of gravity is small suggests that the non-linear diffusion equation approach is applicable to water flow in the matrix in fractured rock masses.

During the initial stage, the matrix absorbed a large fraction of the water flowing through the fracture. After a certain time, most of water came out of the system without being absorbed by the matrix. This outflow would consequently flow into the next system in a real fractured rock mass. The fracture-matrix interaction thus alters the hydraulic properties of a fractured rock mass until some time after the absorption starts. In other words, the fracture-matrix system works as a damper such that a sharp excitation such as an episodic precipitation event results in very high absorption and low outflow. In contrast, the damping effect becomes smaller in the case of a sustained continuous precipitation event. However, this picture can be further modified in a large-scale fracture system, where the aperture variability in natural fractures triggers gravitational instability of an infiltration front within the fracture. In our experiment, due to the limited size and fairly uniform fracture, such features were not observed.

Observation by electron scanning microscopy (SEM) revealed that the *Halomonas* cells attached and grew on surfaces of the mineral grains in this sandstone. No significant difference was observed between the 0.5% and 20% NaCl incubations. The *Halomonas* cells on flat grain surfaces were easily observed, while the cell growth within inter-grain cavities were difficult to identify. Aggregates (micro-colonies) of the attached cells on micro-cracks (grain boundary cracks) were also observed, which corresponds to the initial process of biofilm formation. The *in situ* and *de novo* proliferation of the *Halomonas* cells by binary fission was observed on grain surface.

Biofilms were also formed on wider areas of micro-cracks. The biofilm consisted of loosely interconnected cells, with the cells serving as the initiation cores of biofilm formation and as the pit-fillers of inter-grain cavities. Against the expectation of sheet-like or film-like structure, we have observed non sheet-like bacterial interconnections as the biofilm in most samples. It was thus difficult to determine the hydrophobicity of film-like biofilms by conventional method such as the measurement of contact angle.

The degree of saturation varies over time depending on the rate and duration of the external water supply. It induces saturated-unsaturated cycles in rock masses, which are extreme environments for microorganisms.
Furthermore, the conditions such as unsaturated zones containing methane-free gas, and salinity variation due to the inter-exchange between saline water (sea water extracted due to consolidation) and fresh water due to smectite-illite transformation will be even more extreme environments for microorganisms. Although film-like biofilm was not observed, loosely interconnected cells formed micro-colonies in micro-cracks. This itself may alter hydraulic property of rock matrix. More importantly, it showed a possibility that microorganisms could form biofilms on fracture surfaces and reduce the aperture. This will significantly alter the hydraulic properties of fracture and thus the macroscopic properties of fractured rock mass. Further investigation on the formation of real film-like biofilms over a wide range of hydrophobicities on various rock fracture surfaces, and their effect on the hydraulic properties of rock masses will be needed in the future.

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References
Witherspoon, P.A., Wang, J.S.Y., Iwai, K. and Gale,
亀裂生物圏—水理地質特性と地下微生物活動の相互作用と今後の展望—

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ガスハイドレート安定領域以深では、ガスハイドレートが気体と水に分離し不飽和領域を形成する。いくつかのガスハイドレート帯は沈み込み帯に位置する。そのような場所では、岩盤中の流体移動が水理地質学的な関心の対象となる。不飽和領域は気相・液相が共存する2相流であり、亀裂-マトリックス間で水分のやりとりが発生するため流体移動メカニズムは複雑である。さらに、亀裂表面に形成されると考えられるバイオフィルムにより、亀裂性岩盤の水理特性が変化することが予想される。不飽和亀裂性岩盤の水理特性をより正確に評価するために、単一亀裂を含む岩石ブロックを用いて室内実験を実施した。亀裂中を流れる水分の一部がマトリックスに吸引される現象を実験的に計測し、これを「吸水度」とよばれるパラメータで定量的に評価した。数種の微生物を室内にて培養し岩石表面でのバイオフィルムの形成を試みた結果、亀裂粒子間のマイクロクラックにおけるマイクロコロニーの形成が確認された。理論的考察に基づき、このような微生物バイオフィルムが不飽和亀裂性岩盤の水理特性に影響を与える可能性を示唆した。

キーワード：亀裂性岩盤、不飽和領域、水理特性、亀裂-マトリックス相互作用、バイオフィルム

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