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# Mobility and survival of sulphate-reducing bacteria in compacted and fully water saturated bentonite – microstructural aspects

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# Mobility and survival of sulphate-reducing bacteria in compacted and fully water saturated bentonite – microstructural aspects

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# ABSTRACT

Sulphate-reducing bacteria will not be able to enter MX-80 buffer clay with the intended bulk density, i.e. 1900-2100 kg/m$^3$. Nor will they be able to survive and migrate in such environment.

The only circumstances under which sulphate-reducing bacteria can enter, survive and migrate in engineered soil barriers in a KBS3 repository are those prevailing in backfills with lower MX-80 contents than about 10 % or in more smectite-rich, poorly compacted backfills saturated with electrolyte-rich porewater with Ca as dominating cation. In the phase of hydration and expansion of canister-embedding buffer, bacteria can enter the initially very soft clay gel at the rock/buffer contact to a depth of about a centimeter.

# LIST OF CONTENTS


| | | Page |
|---|---|---|
| 1 | **INTRODUCTION** | 5 |
| 2 | **BUFFER CLAY CANDIDATES** | 6 |
| 3 | **CHARACTERIZATION** | 7 |
| 3.1 | Constitution | 7 |
| 3.2 | Life and growth conditions | 9 |
| 4 | **GEOMETRICAL CONDITIONS** | 11 |
| 4.1 | Bacteria | 11 |
| 4.2 | Clay microstructure | 11 |
| 4.3 | Quantitative characterization of the microstructure | 18 |
| 5 | **CONDITIONS FOR GROWTH AND MOTION WITH RESPECT TO THEIR INTERACTION WITH CLAY** | |
| 5.1 | General | 26 |
| 5.2 | Mechanisms | 26 |
| 6 | **CONDITIONS FOR GROWTH AND MOTION OF BACTERIA WITH RESPECT TO THE ACCESS OF WATER** | 30 |
| 6.1 | General | 30 |
| 6.2 | Access to water | 30 |
| 7 | **EXPERIMENTAL** | 31 |
| 7.1 | Introduction | 31 |
| 7.2 | Dilute suspensions | 31 |
| 7.3 | Dense clay | 33 |
| 8 | **DISCUSSION AND CONCLUSIONS** | 37 |
| 8.1 | Theoretical considerations | 37 |
| 8.2 | Presence of bacteria in buffer and backfill | 37 |
| 8.3 | Experimental validation of the theoretical estimates | 38 |
| 8.4 | Aspects on bacterial survival and migration in backfills | 38 |
| 8.5 | Final statement | 39 |
| 9 | **ACKNOWLEDGEMENTS** | 40 |
| 10 | **REFERENCES** | 41 |

# SAMMANFATTNING

Både teoretiska mikrostrukturanalyser och experimentella undersökningar visar att sulfatreducerande bakterier inte kan penetrera MX-80 buffert med avsedd densitet, dvs 1900-2100 kg/m$^3$. Inte heller är de i stånd att överleva och röra sig i sådan miljö. Skälet är att porstorleken är mycket begränsad och att bakterier binds till leraggregatens ytor vilket starkt reducerar deras rörlighet, och också att de inte förmår förskjuta lermatrisen som de är inbäddade i. Vidare överskrider svällningstrycket avsevärt bakteriernas inre tryck och deformerar och sammanpressar bakterierna. I inledningsskedet av buffertens bevätning kan bakterier nå till någon centimeters djup i den mycket lösa lergelen vid bergkontakten.

De enda villkor under vilka sulfatreducerande bakterier kan tränga in, överleva och vandra i ingenjörsbarriärerna av jordmaterial är de som råder i återfyllningar (backfills) med lägre MX-80 innehåll än ca 10 % eller i dåligt packade, mera smektitrika återfyllningar med elektrolytrikt porvatten och Ca som dominerande katjon. Låg densitet kan härröra från svårigheter att kompaktera. För den avsedda MX-80 halten är inte bakteriell aktivitet möjlig om inte packningsgraden är låg och mättnad skett med saltvatten med Ca som dominerande katjon.

# SUMMARY

Both theoretical microstructural analyses and experimental investigations demonstrate that sulphate-reducing bacteria will not be able to enter MX-80 buffer clay with the intended bulk density, i.e. 1900-2100 kg/m$^3$. Nor will they be able to survive and migrate in such environment. The reason is that the void space is very limited and that adsorption of bacteria on clay aggregate surfaces strongly reduces their capability to move, and also that they are not able to displace the clay matrix in which they are embedded. Furthermore, the swelling pressure will largely exceed the internal turgor pressure and deform and compress the bacteria. At the beginning of the hydration of the buffer, bacteria can enter the very soft clay gel to a depth of about one centimeter from the rock contact.

The only circumstances under which sulphate-reducing bacteria can enter, survive and migrate in engineered soil barriers in a KBS3 repository are those prevailing in backfills with lower MX-80 contents than about 10 % or in more smectite-rich, poorly compacted backfills saturated with electrolyte-rich porewater with Ca as dominating cation. Low density may result from difficulties in compacting backfills. For the intended MX-80 content in backfills, bacterial activity will not be possible except for very poor compaction and saturation with saline porewater with Ca as dominating cation.

# 1 INTRODUCTION

The special microbiology of soils has been in focus of a large amount of scientific investigations in all sorts of soil branches, forestry and agrochemistry. Cell and molecular biology in this huge research field has yielded considerable understanding of the composition and constitution and of the metabolism of a number of organic species, of which sulphate-reducing bacteria are considered to be of particular importance for the longevity of the copper/steel canisters with highly radioactive waste. Also, bacterial dissolution of immobilised radionuclides and production of complexing agents may be of importance for radionuclide migration rates [1]. These issues are dealt with in the present report with special respect to soil physics.

# 2 SCOPE

Microbial activities can have a negative impact on the performance of a repository in several respects. One is that organic debris can form colloids that attach radionuclides escaping from leaking canisters, and migrate through the buffer clay to the surrounding rock and further to the biosphere. Another is direct attack of bacteria on sulphur-bearing minerals in the buffer causing release of sulphide that can attack the copper liner of KBS3 canisters. These matters have been investigated and reported in SKB:s research framework.

The present report is aimed at giving microstructural aspects on the conditions for life and migration of sulphate-reducing bacteria in buffer clay [1]. Such bacteria are believed to be present and active at several hundred meters depth in granite. The species *Desulfovibrio, Desulfomicrobium bacalatum, Eubacterium limosum,* and *Shewanella putrefaciens*, are four common bacteria identified at larger depth in the Äspö underground laboratory, of which the lastmentioned is iron-reducing and hence of less interest in the present context. *Eubacterium* is a representative of anaerobic bacteria of which some form "symbiosomes" of bacteroids in plant roots and others cause animal deseases. None of them is hazardeous to the copper liner or steel core of canisters. However, they may be inherent in bentonites exploited in areas with vegetation and may represent nutrients for other microbial processes. Also, they are examples of the extremely small size that bacteria may have, i.e. down to 0.2 μm.

Focusing on the two firstmentioned groups of sulphate-reducing bacteria one realises the very large number of different species belonging to them, and hence the need for selecting a suitable representative for theoretical and experimental investigation of their mobility and estimation of the conditions for survival in the buffer microstructure. The species *Desulfotomaculum nigrificans,* which is gram-positive and spore-forming, was selected here for this purpose since its nature and properties are similar to those of the sulphate-reducing species identified at Äspö.

# 3 PROPERTIES OF BACTERIA

## 3.1 Constitution

### 3.1.2 Size, shape and density

Cells of the so-called strain *Aspo-2 Desulfovibrio*[1)] bacteria are about 0.5 mm in diameter and 1.7 - 2.5 μm in length. They are equipped with somewhat longer flagellae, which have a cross section diameter of 0.01-0.05 μm. The density of bacteria ranges between 1070 and 1250 kg/m$^3$ [2], commonly averaging at 1100 kg/m$^3$. The species used in the experimental part of the present study *Desulfotomaculum nigrificans* has approximately the same properties.

### 3.1.3 Internal structure

In general, bacteria viewed in cross section appear to be organized into different parts, externally a slime layer surrounding a morphologically differentiated rigid cell wall, which is separated from the internal cytoplasm by a plasma membrane. The outer part of the cell walls are believed to have an outer part exposing polysaccharides and proteins while the inner part (periplasm) consists of peptidoglycan [2]. Depending of whether the bacteria are gram-positive or negative, the outer part is dominated by proteins and various polyalcohols or by polysaccharides. The materials giving rigidity to the cell wall are carbohydrates in the form of polysaccarides with several functional groups like OH and also protons and oxygens exposed on the walls surfaces [3], (Figure 1). The slime coating, which is considered as nonliving secretion or excretion with no active role in the metabolism (all chemical life-associated processes) of bacteria, has a complex composition. In principle, it is made up of polysaccarides but in contrast to the rigid cell wall the slime layer has an amorphous and jelly-like consistency. Staining experiments resulting in coloration of bacteria have indicated the presence of polysaccarides with a characteristic -CHOH-CHOH- group. The cell wall can be considered as a rigid, "mosaic-like" structure [3].

The major constituent of bacteria is water, which makes up 75-90 % of the mass [2]. The organic matter is composed of protein (40-80 %), carbohydrates (1.5-36 %) and lipides (0.4-39 %).

### 3.1.4 Strength and deformability

A spherical shape of bacteria would be expected because of the high surface tension but the obvious deviation from this geometry has been ascribed to the stiffness of the cell wall. The fact that the shape is retained after centrifugation at high speed 400 000 times, implying strong resistance to gravity-induced forces, is

1) Very recently named Desulfovibrio aespoeensis sp. nov. by M Motamedi and K Pedersen (Pers. comm.)

taken as evidence of high strength and elasticity [3]. Slow tension of bacteria shows a strongly ductile, i.e. viscoelastic behavior but dynamic loading by ultrasonic treatment has been reported to yield jagged rupture lines suggesting brittle breakage [2]. The loading rate hence appears to be important for the stress/strain behavior, brittle-type failure probably being caused by the quickly alternating compressive and tension stresses produced by the ultrasonic treatment or possibly by generating an increased internal pressure in the bacterial cell.

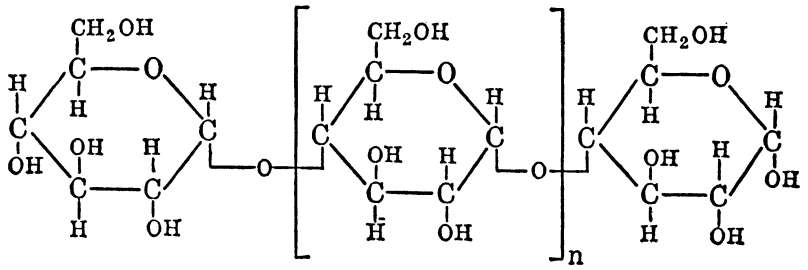


***Figure 1.*** *Polysaccaride molecule.*

### 3.1.5 Charge conditions

Electrophoretic measurements show that bacteria including also the flagella have a net negative charge under normal pH conditions. However, for very high pH reversal to a positive charge has been recorded [2,3]. The charge is due to the surface structure and not to any internal cell charge. The general surface structure, exemplified in Figure 1, means that hydrophilic and hydrophobic groups are exposed in more or less regular patterns.

Suspended bacteria aggregate (agglutinate) spontaneously even by low concentrations of univalent cations or of traces of polyvalent ions. After agglutination bacteria are resuspended only with difficulty. Palygorskite, which is a phyllosilicate clay mineral akin to smectites but with low charge, is known to adsorb bacteria, presumably through van der Waals and hydrogen bonds. This sort of bonding must also be effective when smectite crystals and bacteria come close.

### 3.1.6 Permeability

The application of the theory of osmosis to bacteria requires that water can be transferred into or out of them and it is basic to the function of these microbes that water molecules can move freely through the cytoplasmic membrane, i.e. between its phospholipid molecules. However, many hydrated ions and molecules may not readily migrate through the cell walls [2] and it is believed that while

water on the inside and on the outside forms a continuum it has different chemical compositions. this has implications on the nature of the cell wall. One way of characterizing the wall membrane from the point of permeation is to regard it as a sieve and the capacity of a substance to penetrate it to be a function of the relative molecular volume of the substance and of the voids of the membrane. Early investigations estimated their aperture to less than about 4.5 Å [3], which is compatible with the size of water molecules, but more recently developed models indicate that charge conditions control the permeability [2]. Extensive studies validate that Donnan equilibrium conditions prevail, i.e. that ions of certain charge are excluded [3].

### 3.1.7 Pressure conditions

At equilibrium, the turgor pressure acting in the cytoplasm is of osmotic origin, meaning that any imposed change in volume will change the ion concentration in the interior. The internal osmotic pressure, which can be at least 2.5 MPa [3], acts on the cell wall. In a bacterium located at large depth where high piezometric conditions prevail, the internal pressure must be at least as high in the cytoplasm but the osmotic conditions require that there must also be an internal overpressure. Hence, considering the bacterium to behave as a small element of smectite clay located in the buffer or backfill, one can regard the internal pressure to be the sum of the osmotic pressure, and the pore pressure. The osmotic pressure is, in principle, corresponding to part of the swelling pressure, i.e. the effective pressure, in smectite clay. This latter pressure, which acts on bacteria embedded in the clay matrix, can be several MPa.

## 3.2 Life and growth conditions

### 3.2.1 Nutrients

For the presently discussed bacteria access to organic substance as a source of carbon and energy are required. The latter is provided by plant and animal residue and all sorts of fine organic debris, ranging from bacteria and spongae to humus. The nutrients are brought into the bacteria by the cell membrane transporters or by diffusion into the cytoplasm, which is a colloidal system with 100 to 200 Å particles consisting of i.a. proteins, ribonucleic acid, and phospholipides [2,].

### 3.2.2 Multiplication

Bacteria commonly multiply by binary fission, the cell splitting along the transverse axis [2,3.]. It is manifested by methane production that is reported to be linearly correlated with bacterial growth [1].

### 3.2.3 Pressure conditions

A bacterium will undergo compression and not stay stable in homogeneous smectite clay if the swelling pressure exerted by the clay exceeds the sum of the internal, turgor pressure and the (low) mechanical stress that can be carried by the bacterial body. Since the density and thereby the swelling pressure varies in the clay matrix, there are a number of spots where bacteria may escape from fatal compression.

# 4 GEOMETRICAL CONDITIONS FOR GROWTH AND MOTION

## 4.1 Bacteria

A number of experimental investigations on the survival of sulphate-reducing bacteria have been performed [1] and they indicate that these bacteria can not stay alive when the density of water-saturated MX-80 clay exceeds about 1900 kg/m$^3$. The exact reason for this restriction is not known and the present study is an attempt to identify and quantify the limiting factors.

One condition for survival, growth and motion of *Desulfovibrio* bacteria is their size with regard to the size and connectivity of the voids in the clay matrix. A second factor is the physical condition of the porewater, which is different in differently dense parts of this matrix. A third factor that determines the possibility for bacteria to move is the degree to which they become bound to the clay matrix. A fourth factor is their ability to overcome the mechanical strength of the clay matrix, which is required in order to make them move. All the factors depend on the clay microstructure.

## 4.2 Clay microstructure

### 4.2.1 Components

*Microstructural definitions*

The following terms are used for the various microstructural constituents:

Flake, lamella: Single sheet of smectite crystal lattice.
Stack of flakes: Coherent particle consisting of a number of aligned lamellae.
Aggregate: Coherent group of stacks.
Clay gel: Coherent network of aggregates.
External voids: Air- or water-filled space between stacks of lamellae.
Internal voids: Interlamellar space

*Composition*

Bulk MX-80 contains 65-75 % montmorillonite, 10-14 % quartz, 5-9 % feldspars, 2-4 % mica and chlorite, 3-5 % carbonates and chlorite, and 1-3 % heavy minerals [4].

The chemical composition is as follows [4]:

$SiO_2$ 61-65 %, $Al_2O_3$ 22-25 %, $Fe_2O_3$ 1-7 %, MgO 1-2 %, CaO 0-0.6 %, $Na_2O$ 0-1 %, $K_2O$ 0-3 %

The dominant adsorbed cation is Na (60 %), while Ca represents about 25 % and Mg around 10 %. Fe, Cu, and K are adsorbed to a small extent.

In microstructural modelling it is assumed that montmorillonite makes up 100 % of the minerals and that Na is the only adsorbed cation. The derived models will therefore deviate somewhat from the true microstructural constitution.

*Granulometry*

The grain distribution of air-dry MX-80 clay powder with a water content of 8-12 % is illustrated by Figure 2 [4].

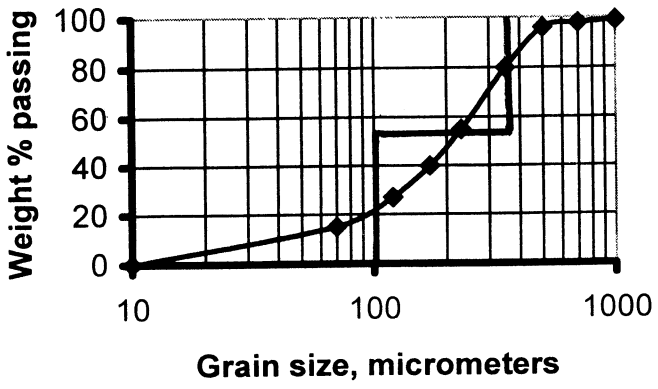



***Figure 2.*** *Grain size distribution of MX-80 clay. The stepped curve represents two grain sizes used in numerical modeling (cf. Figure 4).*

*Crystal structure of montmorillonite*

Definition of the crystal structure of montmorillonite requires that a stack of at least two lamellae of the type shown in Figure 3 is considered [4]. Both models are theoretically possible, the traditional Hofmann/Endell/Wilm version being valid for elevated temperature and adsorption of other cations than Li and Na, while the Edelman/Favejee version may apply to lower temperatures than about 100°C with Li and Na in exchange positions. The latter model implies that more OH-radicals are available than in the firstmentioned model in which OH is exposed only at the particle edges.

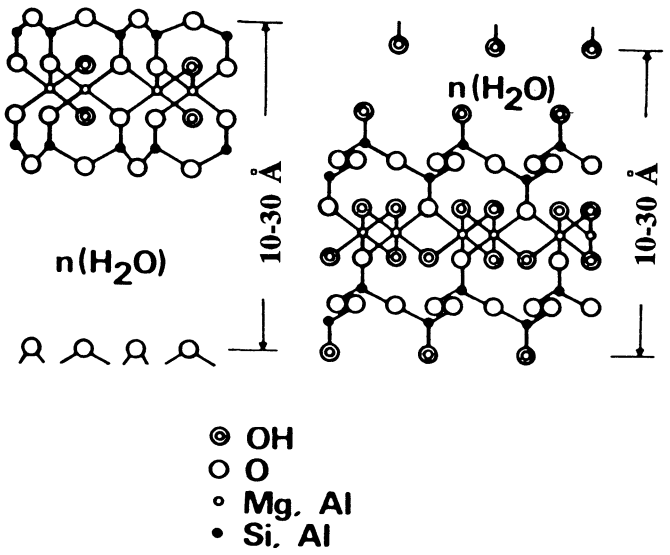



***Figure 3.*** *Crystal constitution models for montmorillonite. Left: Hofmann/Endell/Wilm. Right: Edelman/Favejee [4].*

*Microstructural units*

Most of the smectite stacks in air-dry MX-80 stored at 50-70 % RH, have 1 interlamellar hydrate layer but where Ca and Mg are sorbed there are 2 hydrate layers. When the clay powder is exposed to moist air with RH=100 % or to liquid water, the number of interlamellar hydrate layers increases to maximum 3 when Na is sorbed, while it remains to be 2 when Ca and Mg are sorbed. Table 1 gives estimated basal spacings and average number of interlamellar hydrates for different bulk density intervals.

***Table 1.*** *Number of interlamellar hydrates as a function of Na clay density at water saturation [4].*

| Bulk density at saturation, kg/m$^3$ | Basal spacing, Å | Number of interlamellar hydrates |
|---|---|---|
| 2100-2200 | 13.7-14.6 | 1 |
| 2000-2100 | 14.6-16.5 | 1-2 |
| 1900-2000 | 16.5-18.3 | 2 |
| 1700-1900 | 18.3-19.3 | 2-3 |
| 1500-1700 | 19.3-23.0 | 3 |
| 1400-1500 | 23.0-27.0 | 3* |

* Maximum number of hydrates of intact stack, higher basal spacings indicate presence of more than one stack

### 4.2.2 Evolution of the microstructure of MX-80 buffer clay

*Grain orientation*

The internal structure of MX-80 bentonite grains is inherited from that of the natural bentonite beds. It is characterized by more or less aligned aggregates of stacks, which makes the grains anisotropic. This means that the orientation of the grains affects the behavior of the clay in bulk. Thus, if all grains would be oriented in the same fashion the clay would behave quite differently than if they are randomly oriented.

*Block preparation*

According to a procedure considered by SKB, POSIVA and ENRESA, air-dry clay powder is poured in a form and compacted uniaxially or triaxially under high pressure to form highly compacted blocks. For KBS3 buffer the clay blocks are prepared by compacting the powder under a pressure of 100 MPa. The dry density of the grains is about 1980 kg/m$^3$ for 10 % water content by weight and the dry density of the powder mass poured in the form and slightly compacted is about 1200 kg/m$^3$ (1800 kg/m$^3$ in saturated form). Uniaxial compression causes a reduction in void space between the grains and deformation of the grains that has been calculated numerically assuming the powder grains to behave elastically [4]. Figure 4 shows a scanning micrograph of the largely spherical MX-80 grains.

*Unit cell, compacted state*

Calculation of how the bentonite grains deform and are pressed together to form a cohesive powder mass can be made by taking the initial powder particles to be spherical and make up a unit cell consisting of one big grain and 8 small grains as illustrated in Figure 5. The diameter 0.35 mm of the big grain and 0.10 mm of the small one yield approximately the grain size distribution in Figure 2. The initial void ratio is 1.08 and the initial "effective" porosity 47 %, both being representative of slightly compacted MX-80 powder.

Using appropriate rheological data the uniaxial compression of the unit cell under 100 MPa pressure yields a total uniaxial compressive strain of the model of about 28 %, which is on the same order of magnitude as recorded at practical compression. The net dry density of the model clay is 1850 kg/m$^3$ and the density after complete water saturation 2050 kg/m$^3$, which is representative of SKB buffer clay.

The stress conditions in the grains vary very much. Thus, the average compressive stress exceeds 200 MPa in the interior of the grains while it is zero in the shallow parts (Figure 6). 200 MPa pressure expells water from the interlamellar space to the less pressurized outer parts of the grains, where there are up to 3 hydrate layers in Na clay and 2 in Ca clay.

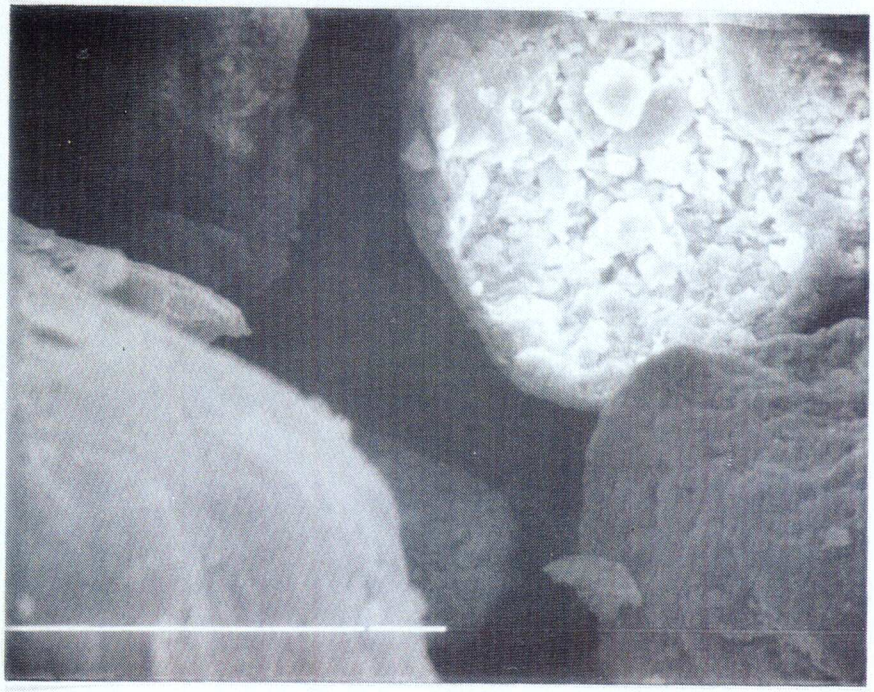


***Figure 4.*** *Scanning micrograph of MX-80 grains. Many of the grains are more or less spherical. The white line is 100 μm.*

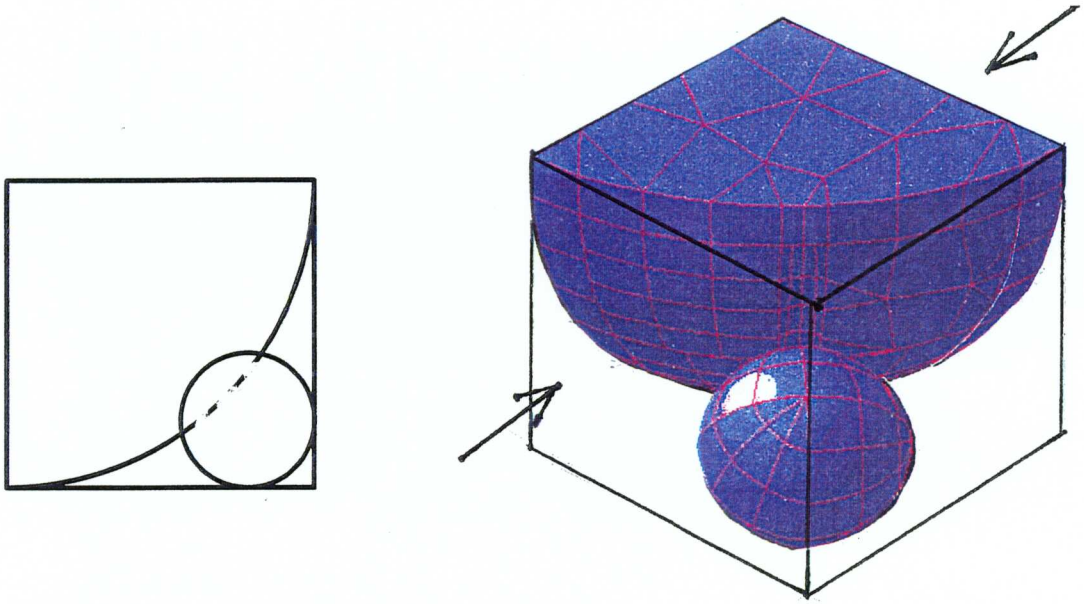


***Figure 5.*** *The basic grain arrangement. Unit cell with 1/8 of big grain (0.35 mm diameter) contained in cubical cell with 0.175 mm edge length. The cell also contains an associated small grain (0.10 mm).*

*Unit cell, hydrated state*

The unit cell is initially air-dry and absorbs water molecules when exposed to vapor or water migrating by flow or in the form of surface diffusion along the grain surfaces. The densest parts of the grains have the highest hydration potential and become wetted quickly if the cell is free to expand, but the hydration is resisted if there is an external pressure or confinement. The rate of water uptake is controlled by the capacity of the surrounding clay matrix to provide water, which means that its hydraulic conductivity is a controlling factor.

The grain growth has been simulated by applying the BEASY code yielding the results in Figure 6 for the case of uniform radial expansion of the grains within the asymmetric unit cell [4]. The swelling process was simulated to be equivalent to thermal expansion, selecting relevant E- and $\nu$-values for the calculation of the distribution of stress and strain on the basis of expansion data and general relationships between bulk density and swelling pressure. This gave E=5 MPa and $\nu$ =0.3 for both Na- and Ca-saturated MX-80. There are open voids in all sections and in the tightest ones the open space consists of three channels with 10-20 μm diameter, while in the most open sections there are channels with larger widths.

While there are no straight open channels between the expanded grains in the clay matrix, tortuous paths are formed by interconnected voids with a diameter of down to 10 μm. A 0.02 $mm^2$ cross section through this model clay with a density at saturation of 2050 $kg/m^3$ contains 3 voids with 10 μm diameter. However, because of the statistical distribution of the grain orientation and because the grains do not make up perfect stacks of smectite lamellae but contain small voids between small stacks, it is expected that there are voids ranging from tens of Å units to tens of micrometers. Also, local large voids are expected. A schematic 2D model is shown in Figure 7.

In practice, the conditions are somewhat different because 20 % of the grains are smaller than 0.10 mm, meaning that the largest voids in the tightest sections after compaction are smaller than 5-10 μm. Another factor is that the grains are in fact anisotropic and hence expand significantly more in one direction than in the others, i.e. almost 3 times more than for grains with randomly oriented stacks of lamellae.This means that where the big grain of the unit cell expands towards the small grain there will be no open space left in cross sections of the type shown in Figure 6, irrespective of the orientation of the small grain. Where the lamination of the small grain is oriented parallel to that of the big grain, the tightness is even higher and expansion to hold more than 1-2 hydrate layers in the stacks will not be possible. This gives a major contribution to the bulk swelling pressure.

Further reasons for an even tighter microstructure is that stacks of flakes become exfoliated from the expanding grains and reorganize to form clay gels in the voids between the grains. Such spontaneous dispersion and reformation of soft gels have been documented by using a humid cell in the 1.5 MV electron microscope of CNRS in Toulouse, France [4,5].

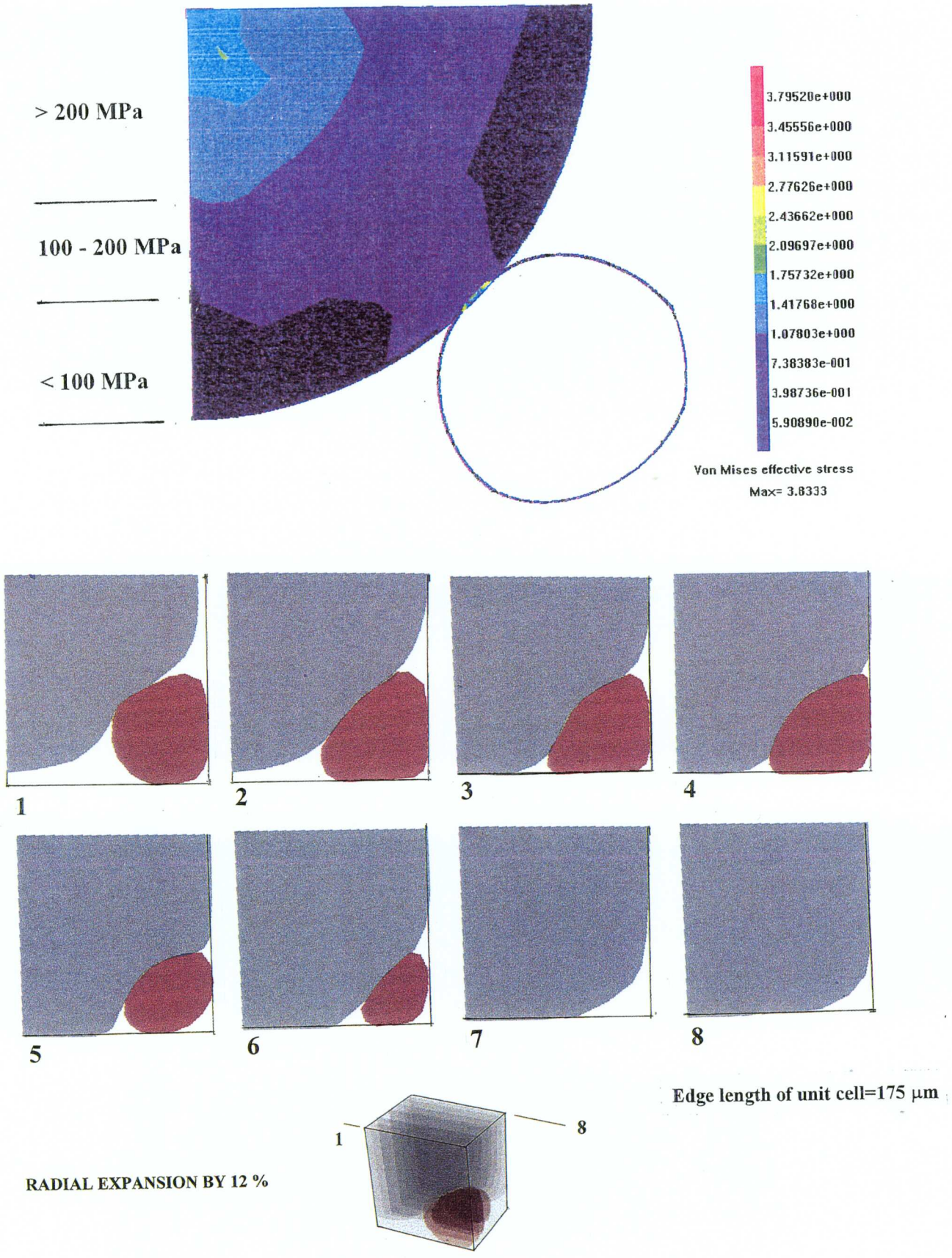



***Figure 6.*** *Grain conditions. Upper: Stress state in the grains after compaction under 100 MPa pressure but before hydration (Mises stress in cross section of unit cell with highest stresses). Lower: Shape of the grains after hydration and expansion. Calculation by use of BEASY. The eight sections are oriented perpendicularly to the direction of compaction of the dry powder.*

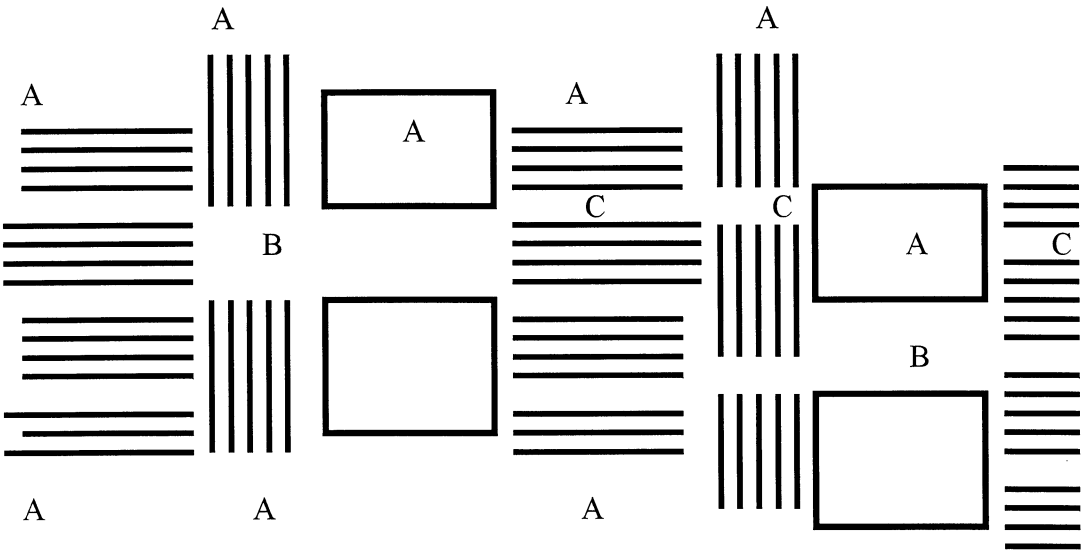



***Figure 7.*** *Schematic microstructural model in 2D of dense montmorillonite clay with orthogonally grouped stacks of lamellae. A) Impermeable stacks of 10 Å lamellae separated by 1-2 hydrate layers. B) Gel-filled very permeable large void. C) Gel-filled poorly permeable narrow void [4].*

A simple estimate is that one third of the grains are oriented such that they completely fill up a cross section through the unit cell after complete hydration. Taking also smaller grains than 0.10 mm into consideration, this is equivalent to a 3D microstructural model consisting of series of coupled cells with one of three cells in each direction being impermeable in this same direction and two cells each containing a void with a diameter of a few micrometers, representing cases with grain expansion perpendicular to the assumed direction as indicated in Figure 7. These voids are expected to be largely occupied by clay gels formed by exfoliated, dispersed and reorganized smectite particles.

## 4.3 Quantitative characterization of the microstructure of MX-80 buffer clay

### 4.3.1 Microstructural parameters

*Definitions*

For the sake of simplicity the clay matrix is defined to consist of two major components:

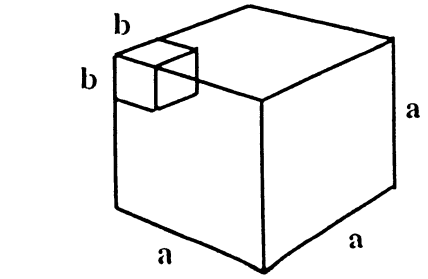
1. Stacks, stack aggregates and non-smectite grains (*a*)
2. Gel-filled voids and unfilled voids (*b*)

This distinction is made on the ground that the firstmentioned component is completely or largely impermeable while the latter offers no or little flow resistance. A further reason is that ion migration in the firstmentioned component takes place by both pore and surface diffusion but almost entirely by pore diffusion in the lastmentioned space. The two microstructural components are related through the coefficients $F_2$ for 2D and $F_3$ for 3D conditions and the ratio depends on the average and individual bulk densities as specified in Figure 8 [4].

*Preparation of micrographs for microstructural quantification*

Microstructural quantification requires access to representative two-dimensional pictures for evaluation of void size and density. Impregnation of the clay must be made for preparing hard specimens from which thin sections can be obtained using ultramicrotomy, atom milling or conventional grinding. The required thickness depends on the purpose of the microstructural investigation [6,7].

It is essential that the preparation does not alter the clay microstructure and bacteria significantly. Minor changes related to a change in basal spacing of stacks of smectite lamellae may not be avoidable but the overall pattern of varying void size and clay density must not be appreciably changed. It is hence needed that the porewater be replaced by an impregnation liquid that can enter and fill the voids in approximately the same fashion as water. A second requirement is that the hardened impregnation material must give the clay a strength that permits sectioning without causing mechanical disturbance.




2D $F_2=b^2/a^2$ $b=a(F_2)^{1/2}$

3D $F_3=b^3/a^3$ $F_3=F_2x(F_2)^{1/2}=(F_2)^{3/2}$ $\rho_{av}=\rho_b F_3 + \rho_a(1-F_3)$

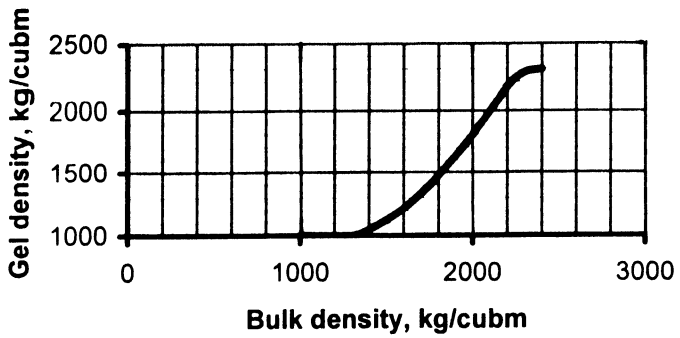



***Figure 8.*** *Microstructural parameters is the average bulk density of the clay and the average density of components* ***a*** *(stacks, stack aggregates and non-smectitic minerals) and* ***b*** *(soft gel fillings and open space). The diagram shows average gel density versus average bulk density.*

Electron transmission microscopy (TEM) of clays based on the experience from biology was introduced about 1960 and its applicability has been assessed and validated on several occasions [4,5,6,7]. For acrylate preparation, which was applied in the present study, the procedure is as follows.

A prismatic specimen with a base area of a few square millimeters and a length of about 10 mm is cut from the water-saturated sample such that its orientation in situ can be defined. It is placed under confined conditions in a rigid cell with filters in a solution consisting of 50 % by weight of ethyl alcohol and 50 % distilled water for 8 hours, followed by emplacement in 90/10 ethyl alcohol/water and 99.5 % ethyl alcohol for 4 hours each. The cell is then transferred to a solution consisting of 85 % by weight of butyl methacrylate and 15 % methyl methacrylate for 4 hours, the process being repeated twice. Finally, the cell with the clay specimen is transferred to a solution of 98 % monomer and 2 % 2.4-dichlorbenzoylperoxide (EMW) catalyst for 90 minutes. Polymerization is obtained by heating to 60°C for 15 hours. Experience indicates that bacteria are preserved in soil impregnated in this fashion [8].

While the traditional acrylate method works well for clays with illite and chlorite as dominant clay minerals, special measures have to be taken when treating expanding clays. Thus, very significant expansion and disintegration take place if the specimens are not effectively confined. Recent investigations have shown that the same structure of compacted MX-80 powder is obtained by saturating the air-dry clay directly with the solution of 98% monomer and 2% catalyst as when following the tedious alcohol/monomer prescription.

*Determination of F-parameters*

$F_2$ and $F_3$ can be evaluated from digitalized TEM micrographs with different degrees of greyness representing different densities. They are converted into different colors for easy interpretation and representation of the variation in density. In the current R&D work this is made by transforming scanned micrographs to digitalized form using the OFOTO 2 code, with subsequent coloring using the GRAPHIC CONVERTER 2.9.1 code on a MacIntosh Power PC6100/66. An example of a processed TEM picture is shown in Figure 9. Using only four colors, clear distinction can be made of parts representing different densities: black parts, i.e. the most electron-absorbing components being the densest parts of component a, and red parts representing relatively dense parts of the same component. Green are soft, porous parts of component *b* while white represent the most porous parts of this component and local open voids.

Depending on the scale, *a* or *b* may dominate and micrographs with an edge length of at least 30 μm appear to be representative for the larger part of the clay matrix.. One gets the approximate relationship between the average bulk density and the gel density (phase *b*) in Figure 10. $F_2$ and $F_3$ are related to the average bulk density of saturated MX-80 clay as shown in Figure 11.

### 4.3.2 Conclusions concerning geometrical restrictions to bacterial survival and activity in compacted MX-80

The space available for hosting bacteria in buffer clay is represented by what is termed the *b* phase in this report, i.e. the gel-filled and locally open voids between denser parts of the clay matrix. Assuming the diameter of the "external" voids, i.e. the pores between the compacted air-dry bentonite grains to be normally distributed, their size is according to Table 2. The density of the clay gels in these voids after hydration is related to the bulk density as shown in the preceding text. It is specified for three reference clays in Table 3. Table 4 shows the average void space in the channels formed by interconnected pores in the gels, assuming that the gel structure is regular orthogonal with the "walls" consisting of 3 lamellae in Na montmorillonite and 10 when the clay is in Ca form.

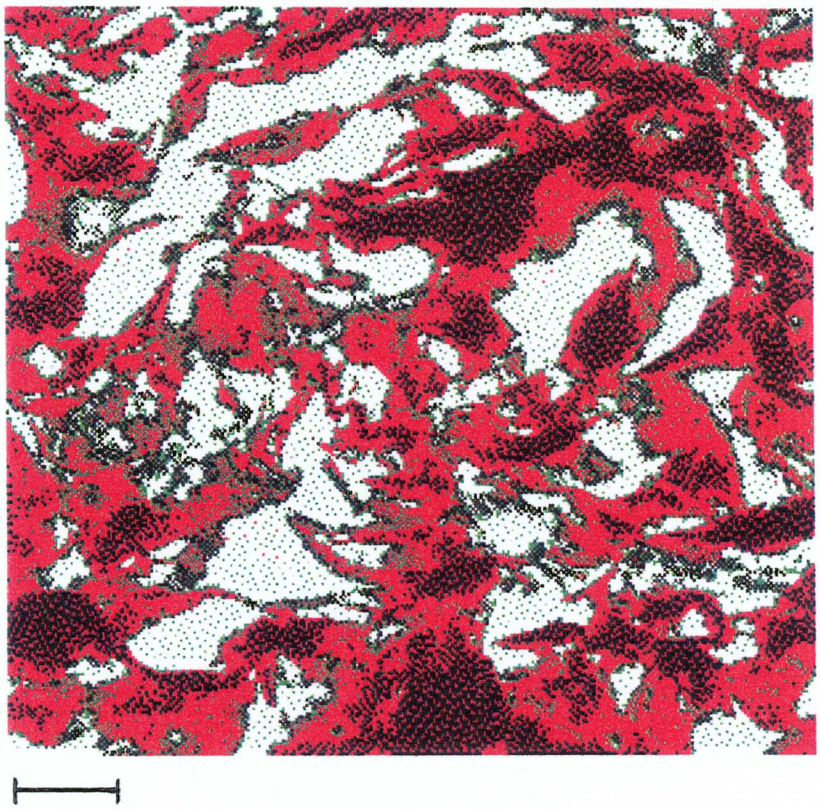


***Figure 9.*** *Example of digitalized micrograph of MX-80 clay with a bulk density at saturation of 1800 kg/m$^3$. Black = densest parts of clay matrix a. Red = relatively dense parts of the same component. Green = soft, porous parts of component b. White representing open parts of this component. Bar is 1 μm.*

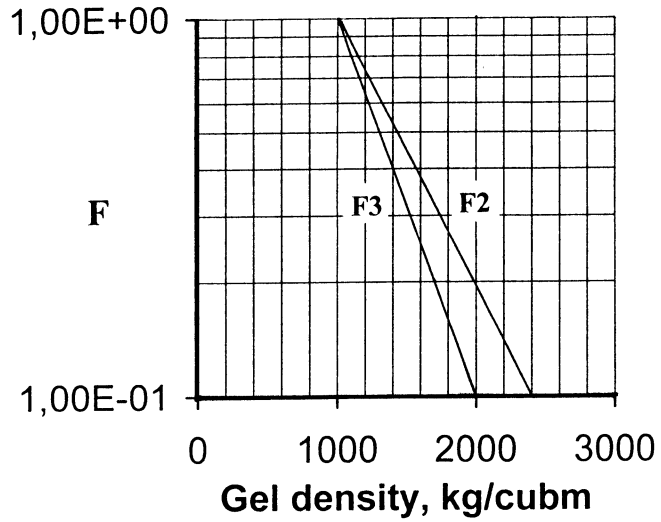



***Figure 10.*** *$F_2$ and $F_3$ versus gel density. Data based on microstructural analysis of artificially prepared MX-80 clay.*

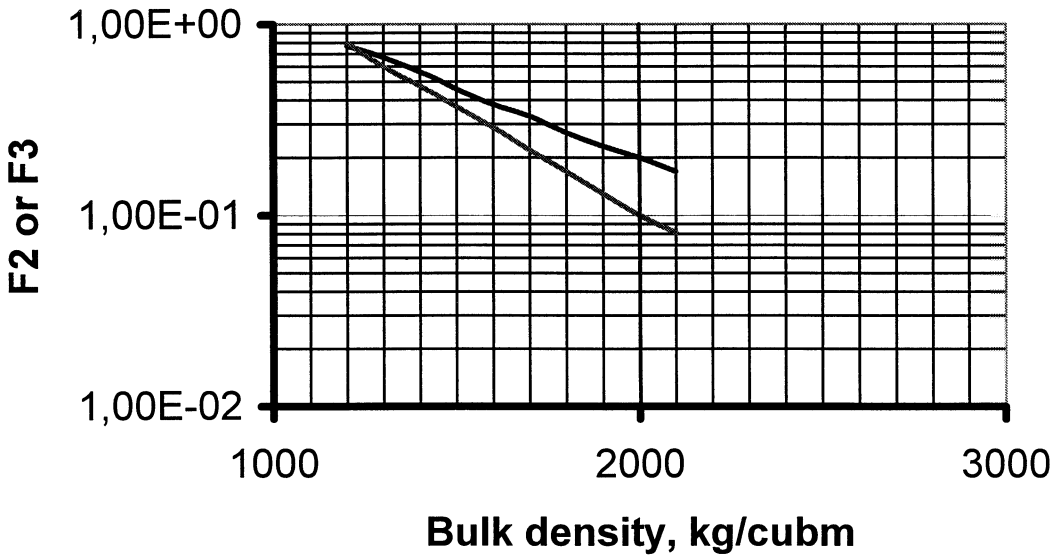



***Figure 11.*** *F2 (upper curve) and F3 versus bulk density. Data based on microstructural analysis of artificially prepared MX-80 clay.*

***Table 2.*** *Size ($D_e$) of "external" voids, i.e. pores between bentonite grains in compacted, air-dry MX-80 bentonite.*

| Clay type | Dry density kg/m$^3$ | Bulk density kg/m$^3$ | $D_e$ μm |
|---|---|---|---|
| A | 1790 | 2130 | 1-3 |
| B | 1350 | 1850 | 1-10 |
| C | 905 | 1570 | 1-20 |

***Table 3.*** *Average density of gel fillings.*

| Clay type | Bulk density kg/m$^3$ | Average gel density, kg/m$^3$ |
|---|---|---|
| A | 2130 | 2000 |
| B | 1850 | 1650 |
| C | 1570 | 1150 |

***Table 4.*** *Average free space in gel fillings.(Channel aperture).*

| Clay type | Bulk density kg/m$^3$ | Average gel density, kg/m$^3$ | Average channel size, Å | |
|---|---|---|---|---|
| | | | Na-form | Ca-form |
| A | 2130 | 2000 | 10-50 | 20-100 |
| B | 1850 | 1650 | 20-100 | 40-200 |
| C | 1570 | 1150 | 30-500 | 200-10 000 |

The channels formed by interconnected gel-filled external voids are assumed to have a varying diameter and to be "crankshaft"-shaped when applying the model in flow calculations and in considering motion of bacteria.

The data in Table 2 suggest that the size of the voids in compacted, air-dry MX-80 clay should be sufficient for survival and motion of *Aspo-2 Desulfovibrio* bacteria when the dry bulk density is lower than about 1700 kg/m$^3$ since they are about 0.5 μm in diameter and less than 3 μm in length.

However, it follows from Table 4 that the openings in the clay fillings of hydrated and matured MX-80 with a bulk density of 2130 kg/m$^3$ (Type A), corresponding to that of the buffer clay, are smaller than 0.01 μm as an average. This space is very much smaller for providing space for *Desulfovibrio* bacteria and this is also the case for MX-80 clay with 1800-1900 kg/m$^3$ density in saturated state, while hydrated clay in Ca form with a density of 1600 kg/m$^3$ has a maximum size of the channels of 1μm (10 000 Å) and may offer sufficient space for hosting this type of bacteria. Still, taking the variation in size along the channels into consideration, the possibility of motion is very limited. Not until the density of saturated MX-80 drops below 1500 kg/m$^3$, which is the case in backfills with a bentonite content of up to 30 %, it is probable that *Desulfovibrio* bacteria can survive and multiply.

While the "external" voids become largely filled with clay gels of varying density some remain open as indicated by examination of ultrathin sections by transmission electron microscopy. Taking the frequency and size of "white" parts of the digitalized colored micrographs as a basis and assuming them to be equally distributed in three dimensions, the size and number of open voids can be illustrated as in Figure 12.

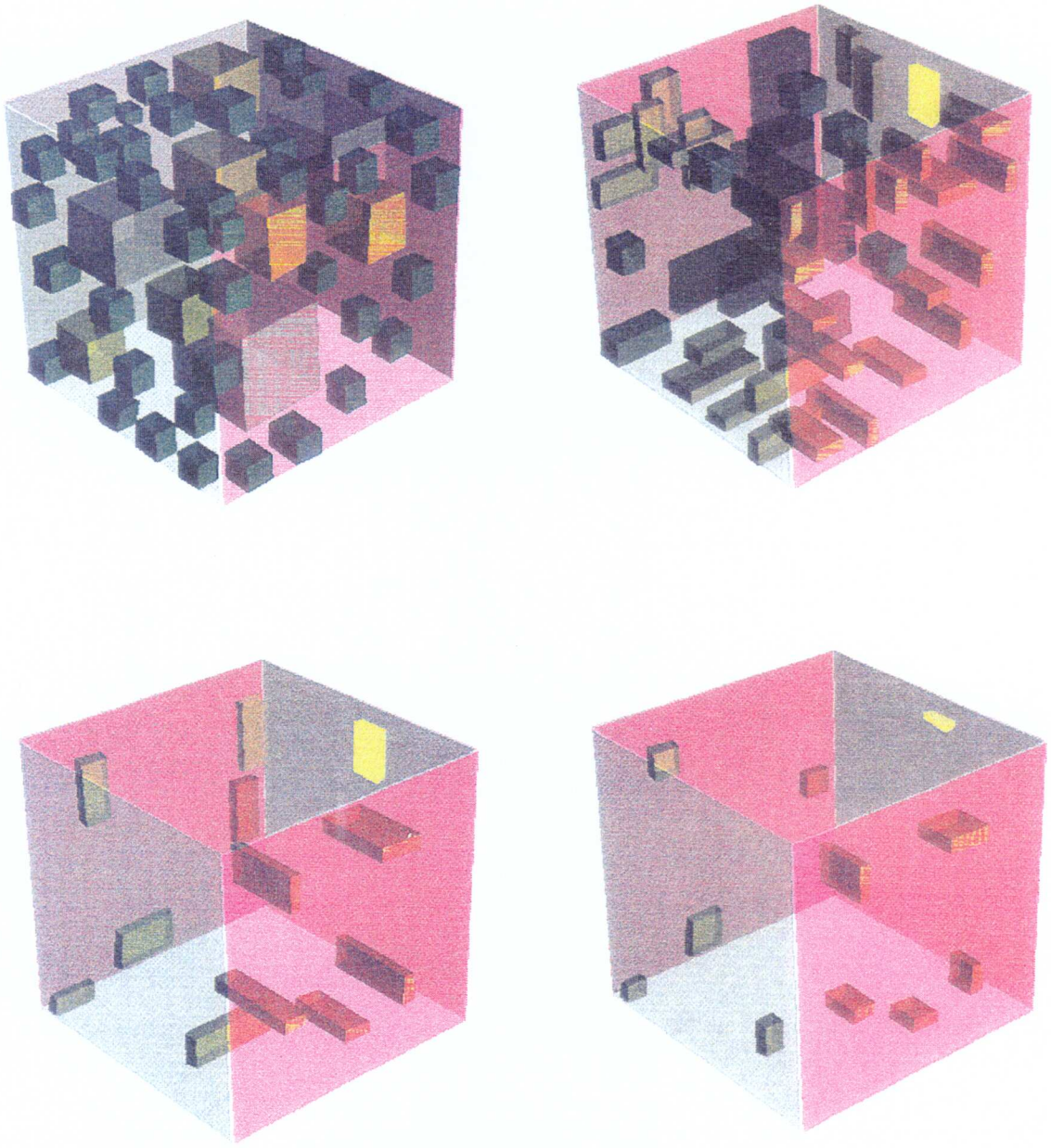


***Figure 12.*** *Distribution of open voids (white in TEM pictures, cf. Figure 9) in cubical clay elements (30 μm edge length) of hydrated MX-80 with different densities. Upper left: Bulk density 1300 kg/m$^3$ (green voids 1-3 μm, yellow 3-5 μm, grey 5-15 μm). Upper right: Bulk density 1600 kg/m$^3$ (green and yellow voids are 3-10 μm long and less than 3 μm wide, grey 5-15 μm). Lower left: Bulk density 1800 kg/m$^3$ (all voids less than 10 μm long and 1-3 μm wide). Lower right: Bulk density 2000 kg/m$^3$ (all voids less than 5 μm long and less than 2 μm wide).*

Figure 12 shows water saturated MX-80 clay with different densities. At a density of 1300 kg/m$^3$ the clay contains numerous more or less isodiametric, gel-filled "external" voids with a size 3-10 μm in which bacteria can be hosted if they are able to displace the gel. For a bulk density at saturation of 1600 kg/m$^3$ the number is smaller and the void shape elongated with the smallest diameter being about 3 μm, which offers smaller but sufficient space for a certain small number of the bacteria considered. However, since the clay gel in the voids is denser, the bacteria need to be able to push the clay aside. For 1800 kg/m$^3$ bulk density large open voids are much less frequent and their size smaller, and, consequently, the density of the gel fillings higher. Finally, for a bulk density of 2000 kg/m$^3$ the number and size of "external" voids are even smaller and hardly any bacteria will be retained in the dense gel fillings without undergoing compression.

# 5 CONDITIONS FOR GROWTH AND MOTION OF BACTERIA WITH RESPECT TO THEIR INTERACTION WITH SMECTITE CLAY GELS

## 5.1 General

A major point is the condition under which bacteria enter the buffer or backfill. Sulphate-reducing bacteria are not assumed to be contained in the clay powder but have to enter the buffer or backfill from the surrounding rock in the course of or after complete hydration for reducing sulphates. One can imagine two cases; i.e. when bacteria move into unsaturated buffer or backfill which are subsequently hydrated, and when they move into fully saturated materials. In the first case it is a matter of survival of bacteria already in the clay soils when they become wetted, while in the second case the issue is if bacteria can move into hydrated soils.

The possibility of survival of bacteria in unsaturated clay that is subsequently hydrated primarily primarily depends on their ability to resist desiccation and the swelling pressure. Secondarily, it depends on the ability of survived bacteria in the expanded smectite to obtain nutrients without which they turn into a dormant state and finally die. The mobility is hence of major importance both because it is required for long term survival of initially survived bacteria and for the sulphate reduction that is of concern. We will confine ourselves here to consider bacteria in fully hydrated and matured clay.

Hindrance of growth and motion of *Aspo-2 Desulfovibrio* and related bacteria takes place by the following mechanisms:

1. Adsorption of bacteria on stacks of smectite lamellae at the boundaries of channel voids.

2. Anomalous viscosity of porewater.

3. Strength of the clay gels in the "external" voids.

## 5.2 Mechanisms

### 5.2.1 Adsorption of bacteria

Coupling of the walls of bacteria and smectite stacks occurs by establishment of van der Waals forces and by hydrogen bonds between surface-active functional groups like carboxyls (COOH), hydroxyls (OH), carbonyls (C-O) and quinone (C=O), [8]. Coulomb bonding can also be assumed to take place between positively charged edges of smectite stacks and negatively charged bacteria, and, like in aggregation of clay particles, by polarization of edge-adsorbed polyvalent cations [9].

The strength of the bonds between smectite clay stacks and bacteria is estimated to be on the same order of magnitude as between aggregated clay particles. Since dispersion of such aggregates require rather intense agitation by ultrasonic treatment, the sorption is assumed to prevent separation by other means than rather strong water flow. Additional support to this belief is the aforementioned adsorption of bacteria by clay minerals of similar type (palygorskite).

The probability of catching of bacteria that are free to move in clay void space depends on their mode of motion. In an environment that is uniform with respect to nutrients the motion of bacteria is more or less random [2,3], which means that they will ultimately be caught by the clay. If nutrients are available in the form of inherent humic substances, they are attached to the clay and the bacteria are apt to direct their movement to the clay/organics and become caught also in this case.

### 5.2.2 Viscosity of porewater

In the dense parts of the clay matrix, represented by the *a*-phase, the fraction of interlamellar, largely immobile water dominates and the resistance for bacterial motion is very high. In the *b*-phase this fraction is smaller but it is still 30-50 % of the total porewater mass in clay gels with a density of 1650 kg/m$^3$ (dry density 1000 kg/m$^3$, cf. Figure 13) which corresponds to a bulk density at saturation of 1850 kg/m$^3$. When the clay gel density at saturation drops to about 1150 kg/m$^3$ (dry density 240 kg/m$^3$), which corresponds to a bulk density at saturation of 1570 kg/m$^3$, the fraction of immobile water is only about 10 %.

These figures show that the content of largely immobile water in buffer clay is very high. However, the water in the clay gel channels has the same viscosity as bulk water except within 5-10 Å distance from the channel walls of clay mineral particles. Hence, the only possible space for bacteria motion is in the channels in clay gels and in isolated open voids.

### 5.2.3 Strength of the clay gel

*General*

Bacteria locked in clay gel channels and isolated voids may be able to make their way by displacing the gel, which requires either that they exert a sufficiently high pressure on the clay gel, or that they produce tensile failure by penetrating into it. For plant cell growth in the form of elongation of the cell wall the required energy is provided by the turgor pressure produced by production of intracellular hormones [3], while for bacteria, this pressure is caused by production or uptake of osmolytes [2].

*Displacement by expansion*

The criterion for a bacterium to survive and move is primarily that the pressure acting in the cytoplasm of a bacterium is at least as high as the swelling pressure exerted by the surrounding clay. This pressure is caused by phase $a$ which can be calculated by use of the microstructural parameter $F_3$ [4].

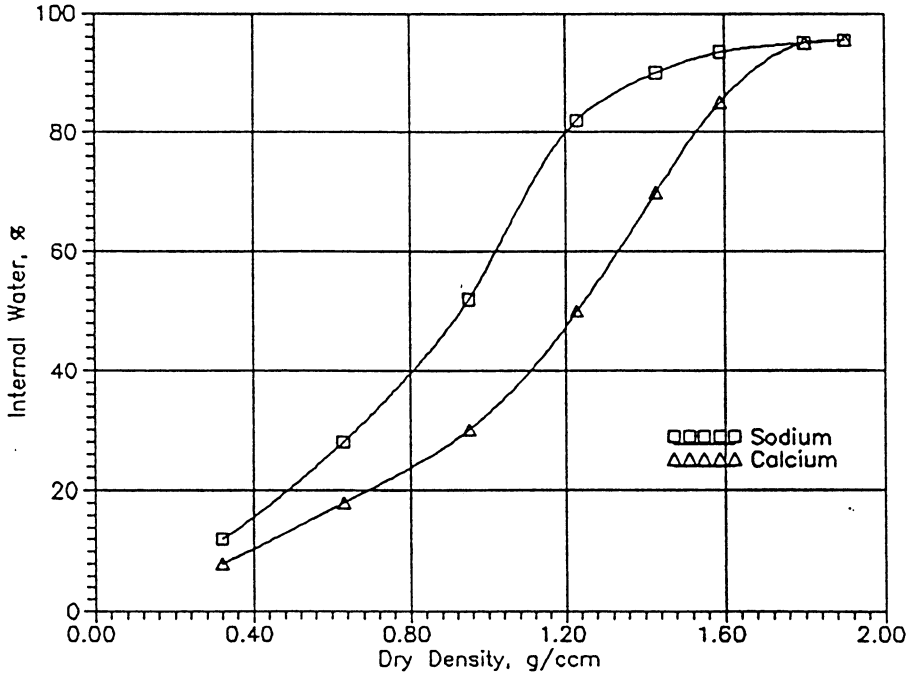



***Figure 13.*** *Theoretical relationship between dry bulk density and content of interlamellar water expressed in percent of the total porewater content.*

The swelling pressure is proportional to the product of the true swelling pressure of the pressure-controlling component $a$ and the volume ratio $(a^3\text{-}b^3)/a^3$. This ratio is $(1\text{-}F_3,)$, which specifies the volume fraction of this component. For the bulk density 2130 kg/m$^3$ $\rho_a$ is 2150 kg/m$^3$ and the swelling pressure of this dense component about 10 MPa assuming isotropic distribution of the orientation of smectite lamellae and Na as adsorbed cation [4]. For the bulk density 1850 kg/m$^3$ the density of the massive part of the clay matrix is 1900 kg/m$^3$ yielding 1.5 MPa swelling pressure. For the bulk density 1570 kg/m$^3$ the gel density is 1750 kg/m$^3$ and the swelling pressure of this fraction about 0.3 MPa. For Ca clay the pressure is almost the same as for the Na state when the bulk density is 2130 and 1850 kg/m$^3$, respectively, while experiments show that it is only about 0.02 MPa for the bulk density 1570 kg/m$^3$.

Knowing the turgor pressure of the bacteria one can conclude whether they can overcome the clay swelling pressure and displace the clay gels. Bacteria with a turgor pressure of 2.5 MPa, which has been reported in the literature [3], would make them able to penetrate MX-80 clay with a bulk density of about 1900 kg/m$^3$ but it is not known whether *Desulfovibrio* belongs to this category.

*Displacement by fissuring*

Another way of estimating the potential of bacteria to penetrate the clay matrix is to assume their propagation to be akin to gas penetration. Gas moves in "paths of least resistance" represented by interconnected open voids or gel-filled channels with lower density than the rest of the clay matrix. According to Harrington and Horseman [10] gas penetration requires separation of dense matrix components ("fracturing"), which implies that the critical pressure for penetration is close to the bulk swelling pressure for MX-bentonite. This is tentatively demonstrated for high bulk densities and moderate rates of pressure increase. Assuming this mechanism to be responsible for gas penetration also for lower densities and applying the hypothesis to bacterial penetration, the same conditions prevail as for displacement of the gel by bacterial expansion. It means that the same criterion applies as for the aforementioned "displacement by expansion".

# 6 CONDITIONS FOR GROWTH AND MOTION OF BACTERIA WITH RESPECT TO THE ACCESS TO WATER

## 6.1 General

As to the importance of water, the ability of bacteria to survive depends on the access to water while their mobility is affected by the viscosity, which is different in differently dense parts of the clay matrix.

## 6.2 Access to water

The access to water depends on its physical state, which is often expressed in terms of activity and which is determined by the hydration (suction) potential of the clay and the bacteria. The suction potential of smectite is tremendous as shown by measurements; it is on the same order of magnitude as the swelling pressure but with inverted sign. In fully water saturated smectite clay of MX-80 buffer type with a bulk density of about 2000 kg/m$^3$ the large majority of the porewater is in interlamellar positions and strongly held there by interlamellar cations and/or the clay lattice (cf. Figure 13). This water is not accessible to bacteria, but since the water in the softer gels is in free condition, they may utilize it for avoiding desiccation.

***It is concluded from this that critical conditions for survival, life and multiplication of bacteria in water saturated buffer clay is not the access to water but the swelling pressure and mechanical hindrance of motion by the clay matrix.***

# 7 EXPERIMENTAL

## 7.1 Introduction

Experiments were performed with MX-80 clay and *Desulfotomaculum nigrificans* (DSM=574) for determining the mobility and condition of bacteria in buffer clay with different densities. Light microscopy was made of dilute suspensions of clay in distilled water and a calcium chloride solution to which a bacteria suspension had been added. Scanning microscopy was made on samples with varved clay and bacteria layers after water saturation and rest.

## 7.2 Dilute suspensions

### 7.2.1 Procedure

The scope was to find out if the bacteria were preferentially sorbed by the clay or if they stayed in the voids of the clay gel. The procedure was to prepare stable clay gels with a density of 1100-1300 kg/m$^3$ by mixing MX-80 clay and distilled water, and a semi-stable gel with 1500 kg/m$^3$ by mixing MX-80 clay and 3.5 % $CaCl_2$ solution, add to them drops of bacteria solution with a concentration of 3.8E+8, and then observe where the bacteria were located using a magnification of 1000 x. The bacteria suspensions were colored with acridine orange according to a standard procedure so that they could be seen in fluorescent light. Cover glasses were applied for protecting the samples.

### 7.2.2 Results

Scanning was made over the larger part of the sample surfaces to identify the location and motion of bacteria, which appeared bluish while the clay matrix had a grey/yellow/brown color. The porewater appeared black.

The investigation was initiated by studying very dilute systems, i.e. gels with a density of 1001 to 1005 kg/m$^3$ prepared with distilled water, and with a density of 1005 to 1010 kg/m$^3$ prepared with 3.5 % $CaCl_2$. Examination of the clays with distilled water showed that the bacteria were all caught and immobilized in the clay matrix (Figure 14), which had voids with a size of up to 10 μm. In the calcium chloride solution with a density of 1010 kg/m$^3$ the bacteria appeared to be free and largely contained in the water between the clay aggregates, which did not form a stable gel (Figure 14). Since it was not expected to find mobile, unassociated bacteria in clay with higher densities than those which yield void sizes smaller than 10 μm, no further experiments of this sort were made.

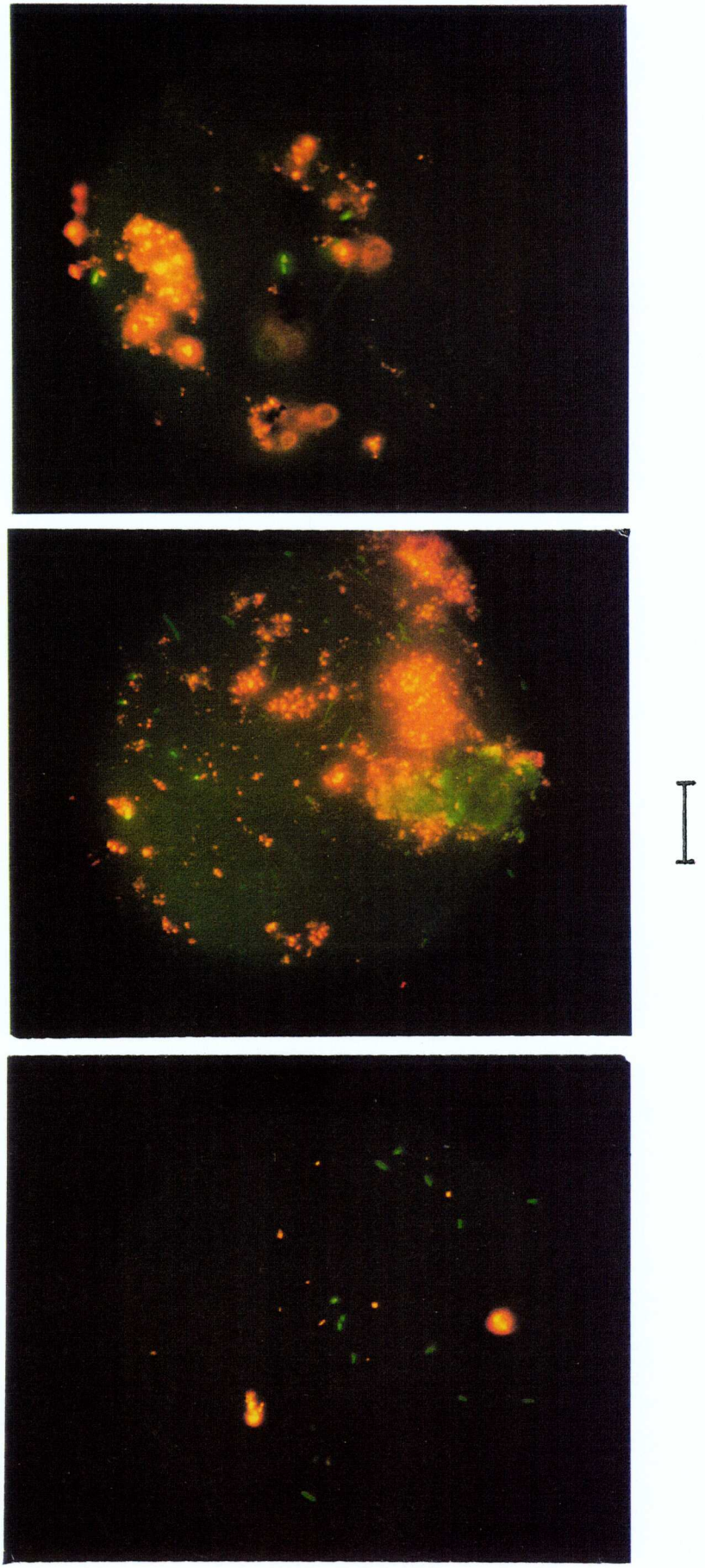


***Figure 14.*** *Bacteria in dilute clay. Upper: bluish bacteria as single objects attached to small clay aggregates in distilled water. Central: Large bluish/greenish bacterial cluster integrated in clay aggregate in distilled water. Lower: Discrete bluish bacteria dispersed in large void of Ca-clay. Bar=10 μm.*

## 7.3 Dense clay

### 7.3.1 Procedure

Samples of sandwiched dense MX-80 clay and bacteria were prepared in cells with 10 mm diameter and 10 mm height by layerwise application of MX-80 powder on which drops of suspensions with a concentration of 3.8E8 bacteria per milliliter. The applied material was compacted in the course of the filling operation to densities that would correspond to 1500 and 2100 kg/m$^3$ after complete saturation with distilled water. The clay powder had been stored in RH about 100 % several weeks before the preparation, yielding a water content of about 20 %, which implies that the interlamellar space was fully saturated and the water tension hence very low. The bacteria should therefore not suffer from dessication. Furthermore, the compacted samples, which were confined between filters in the cells, were contacted with water for improved water saturation and for providing possibilities for the bacteria to move into the clay layers.

After one week the cells were placed in an oven at 100°C for a few hours and then partly saturated with the solution of 98% monomer and 2% catalyst described earlier in the report. Following polymerization at 60°C for 24 hours, the samples were exctracted and prepared for scanning electron microscopy. This work was performed by Dr Jörn Kasbohm, University of Greifswald, Germany.

### 7.3.2 Results

The specimens were fractured along planes perpendular to the varved structure and scanned in order to find the original bacteria-rich layers, and bacteria that could have moved into the initially bacteria-free clay layers.

*Clay with a bulk density corresponding to 1500 kg/m$^3$ at complete water saturation*

Despite very careful examination of more than 100 micrographs very few bacteria could be identified and they were all located where the bacteria-rich suspensions had been applied.

Figure 15 shows micrographs and clarifying scetches of clay elements containing bacteria. The pictures show that they are tightly embedded in the clay matrix with apparently no possibility to move. The bacteria give the impression of being free over part of their length but this is because the fracturing of the clay happened to take place at these sites. Still, they were obviously stronger than the clay matrix and could resist the tension force that broke the matrix, hence indicating that the adhesion between bacteria and clay was not as strong as the cohesion of the clay. The bacteria were found to be oriented parallel to the varves.

An important observation is that the bacteria retained their ellipsoidal shape, which, together with the fact that they did not fracture, demonstrates a high

strength. The bacteria obviously resisted the swelling pressure of the clay, which averaged at 200 to 300 kPa [4]. This means that the internal turgor pressure balanced or exceeded this pressure.

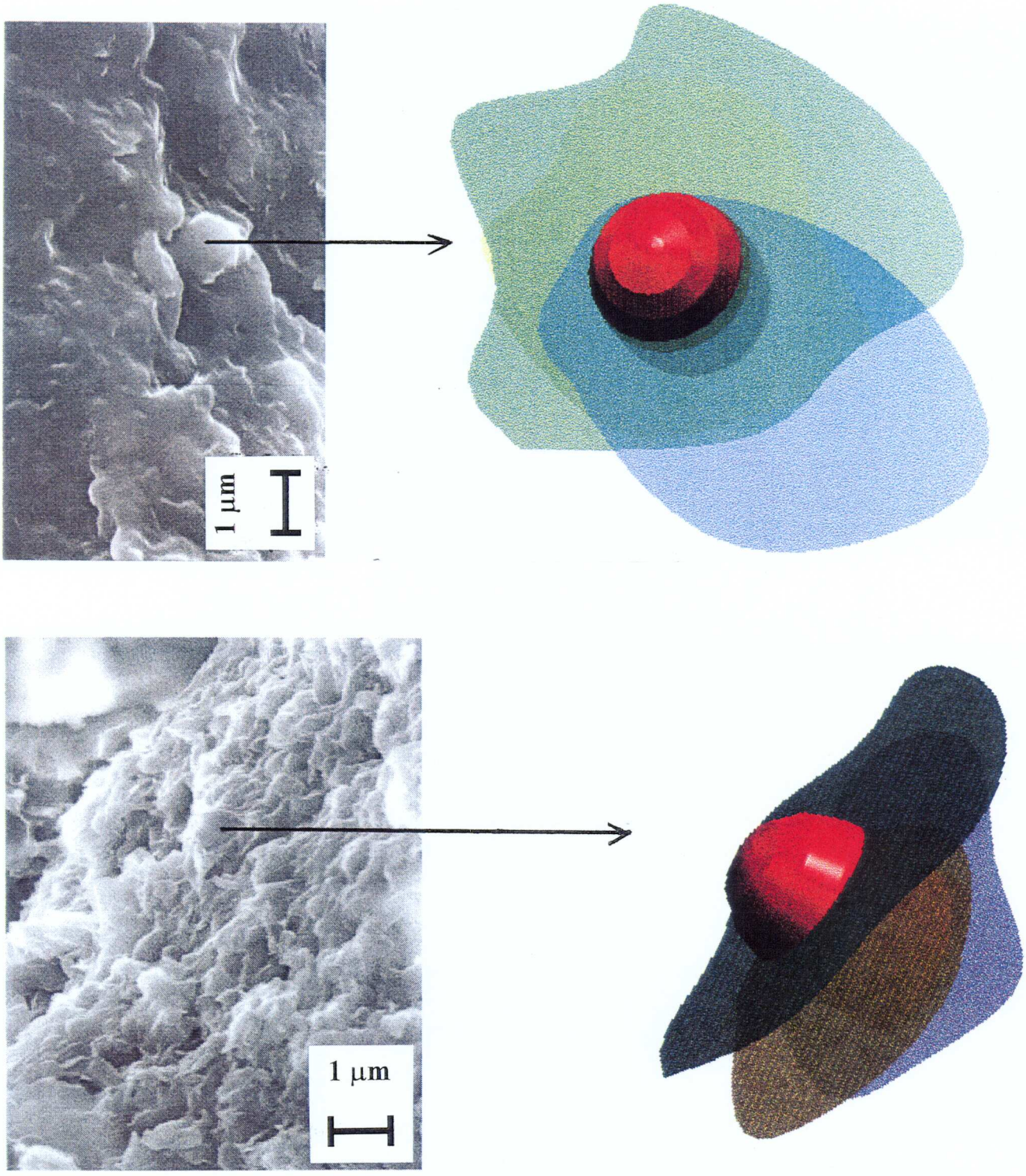



***Figure 15.*** *Examples of identified bacteria embedded in clay matrix of soft MX-80 buffer clay. Red: Bacterium. Green: Clay matrix.*

*Clay with a bulk density corresponding to 2100 kg/m$^3$ at complete water saturation*

The presence of bacteria in the dense samples was even harder to detect than in the soft ones. More than 100 micrographs were examined with utmost care but no very clear proof of bacteria could actually be found. Figures 16 and 17 show possible examples of distorted bacteria that appeared on surfaces exposed by the fracturing involved in preparing specimens for scanning microscopy. EDX cannot be used for safe identification of organic species and the true nature of the objects is therefore hypothetic. However, the absence of visible bacteria demonstrates that they must have been effectively integrated in the clay matrix and distorted under the prevailing swelling pressure of about 10 MPa [4], which they obviously did not sustain.

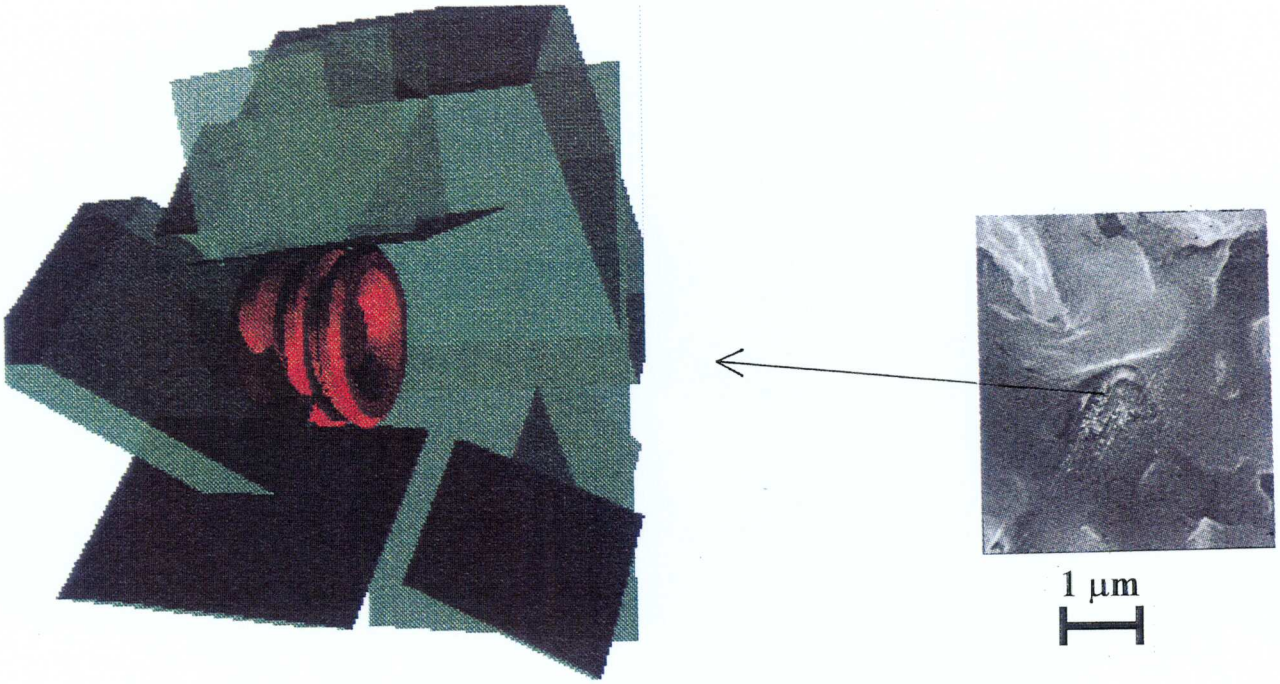



***Figure 16.*** *Example of identified bacteria in dense MX-80 buffer clay (Red: Bacterium. Green: Clay matrix). The micrograph is assumed to show a strongly compressed bacterium partly covered with slime.*

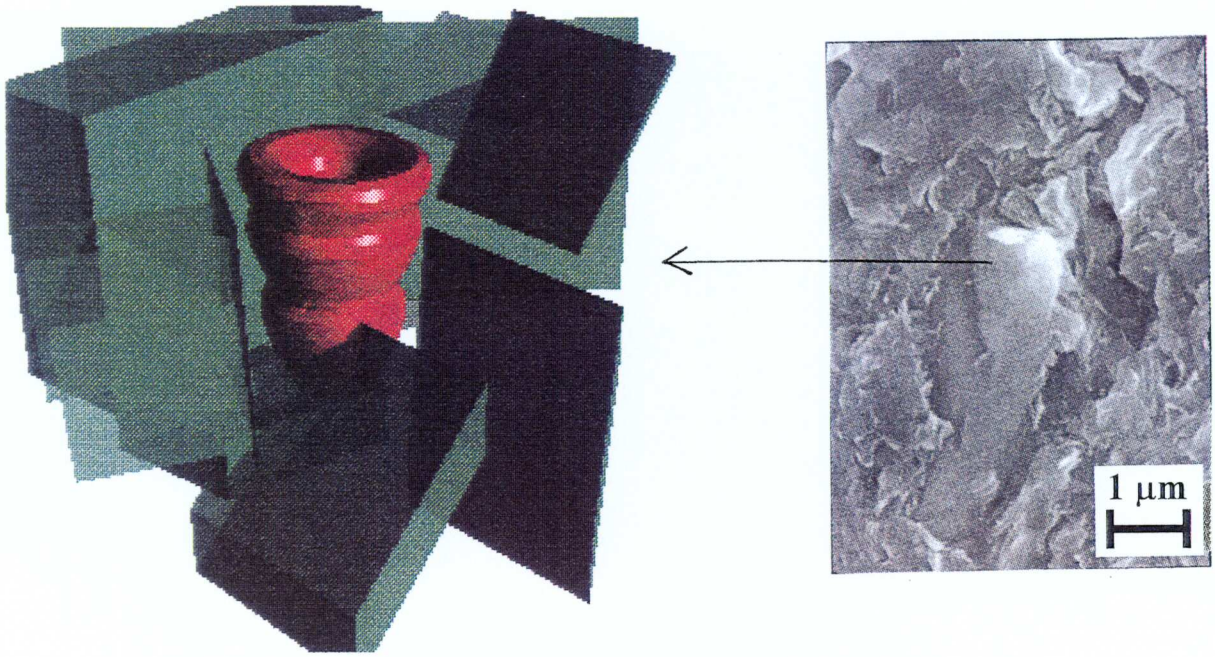



***Figure 17.*** *Example of identified bacteria in dense MX-80 buffer clay (Red: Bacterium. Green: Clay matrix). The micrograph is assumed to show an axially compressed bacterium partly covered with slime. The schematic drawing shows less skewness than exhibited by the micrograph.*

# 8 DISCUSSION AND CONCLUSIONS

## 8.1 Theoretical consideration

Estimation of the void size in MX-80 clay based on comprehensive microstructural analyses and theoretical modeling shows that the space available for hosting sulphate-reducing bacteria is limited to the softest parts of the clay gels that occupy voids between denser aggregates, and to local gel-free voids in the clay matrix.. Theoretically, there are very few voids that offer sufficient space in clay buffer with a bulk density at water saturation higher than about 1500 kg/m$^3$ when the electrolyte content is low or moderate. In clay with very salt porewater and Ca as dominating cation, the voids are sufficiently large to provide sufficient room. The assumption of a density equal to 1500 kg/m$^3$ will not be relevant to matured buffer clay in bulk but it may be representative of soft parts of the clay gel matrix and also of the clay component of mixtures of MX-80 and ballast [11]. It should be added that bacteria can enter the very soft clay that is formed between rock and buffer before the latter has hydrated significantly. Bacteria, which will subsequently be trapped and immobilized, are hence expected to be found in the buffer within about one centimeter from the rock contact at least if Ca is the dominant cation species in the groundwater. Spores may be found as well.

In MX-80 buffer clay with a density exceeding 1800-1900 kg/m$^3$ the frequency of voids with sufficiently large size to host bacteria is insignificant. While certain bacteria may still be located in voids in the softest clay gels where they have access to free water and escape the high swelling pressure, they will not be able to migrate through the clay because of adsoption on clay and because they will not be able to exert sufficient pressure or driving force for moving through the gels. This means that nutrients will not be available, which brings them into a dormant state and ultimately will cause their death. Spores will not be produced in the fatal stage and they are estimated to be adsorbed on the confining clay of living bacteria for the assumed density interval.

In MX-80 buffer clay with a density ranging between 1500 and 1800 kg/m$^3$ the frequency of voids with sufficiently large size to host bacteria is limited. Certain bacteria may be located in voids in the softest clay gels where they have access to free water and escape the high swelling pressure, but they will not be able to migrate through the clay because of adsoption on clay surfaces.

## 8.2 Presence of bacteria in buffer and backfill

It follows from the decribed theoretical estimates that bacteria are not believed to be able to migrate in MX-80 buffer clay with higher bulk density at water saturation than around 1500 kg/m$^3$ and they are definitely prevented from moving into and through buffer clay of normal density, which is higher than 1800-1900 kg/m$^3$. This means that attack on minerals or canisters by bacteria entering from rock fractures is not possible. Only bacteria that are initially present in the clay

powder used for preparing buffer and backfills would have a chance to appear in these barriers but the density, which determines the void geometry, will make them unable to be active in buffer clay of MX-80 type with the planned bulk density.

***The only possible case of bacterial activity in matured MX-80 clay would be represented by tunnel and shaft backfills with a density of the clay component lower than about 1500 kg/m$^3$. This issue is discussed in a subsequent section.***

## 8.3 Experimental validation of the theoretical estimates

The experiments performed fully support the theoretical predictions. Thus, for a bulk density of about 1500 kg/m$^3$ bacteria of the investigated sort appeared to be effectively embedded and confined by clay gels that prevent them to migrate or even survive. For a bulk density of around 2100 kg/m$^3$ the swelling pressure of bacteria would theoretically cause strong deformation by compression and this seems to be illustrated by the few examples that are assumed to have been found in a dense clay sample.

The study of bacteria mixed with very soft clay gels demonstrated that bacteria are adsorbed on the surface-active smectite aggregates and immobilized in low-electrolyte porewater at bulk densities of 1100 kg/m$^3$, while they seem to be free to move in water with 3.5 % $CaCl_2$ solution even at a bulk density of 1500 kg/m$^3$. This is in agreement with the theoretical considerations as well.

## 8.4 Aspects on bacterial survival and migration in backfills in shafts and tunnels

The criteria for survival and migration of bacteria that the swelling (effective) pressure must be very limited and the clay gel density very low, may be satisfied by backfills consisting of mixtures of MX-80 clay and rock ballast. Preceding investigations have shown that such mixtures, in contrast to backfills of natural smectitic clays like the Friedland Clay, may contain soft clay gels and unfilled voids if the content of MX-80 clay and the bulk density are low [11]. Thus, for 10 % by weight of air-dry MX-80 and a bulk dry density of 1750 kg/m$^3$ (2100 kg/m$^3$ at water saturation), the average density of the clay component is 1540 kg/m$^3$ and it is probable that there are continuous clay gel channels with a density down to 1300 kg/m$^3$ [4]. If the porewater salinity is high and Ca the dominant cation, it is possible that sulphate-reducing bacteria of the considered type can survive and migrate in this sort of backfill.

For backfills with 30 % MX-80 and compacted to a dry density of 1750 kg/m$^3$, which requires much more compaction energy than preparation of mixtures with 10 % MX-80, the average clay density is 1890 kg/m$^3$. They are not expected to contain continuous clay gel paths with an average density lower than 1600 kg/m$^3$,

but the density may locally be as low as 1300-1500 kg/m$^3$ [4,11]. Hence, it is believed that dense backfills with 30 % MX-80 or natural smectitic clays like the Friedland clay will not offer conditions for practically important bacterial survival and migration. However, if the dry bulk density is lower than about 1500 kg/m$^3$ (1950 kg/m$^3$ at water saturation) such conditions may prevail if the porewater salinity is high and Ca the dominant cation.

## 8.5 Final statement

1. Sulphate-reducing bacteria will not be able to enter MX-80 buffer clay with the intended bulk density, i.e. 1900-2100 kg/m$^3$. Nor will they be able to survive and migrate in such environment. Before the buffer blocks have hydrated and expanded in the deposition holes, bacteria can enter the very soft clay that is formed between rock but they will be subsequently be trapped and immobilized deeper than about 1 cm from the rock contact.

2. The only circumstances under which sulphate-reducing bacteria can enter, survive and migrate in hydrated engineered soil barriers in a KBS3 repository are those prevailing in backfills with lower MX-80 contents than about 10 % or in more smectite-rich, poorly compacted backfills saturated with electrolyte-rich porewater with Ca as dominating cation. Low density may result from difficulties in compacting backfills.

3. Bacteria may contaminate the clay component of backfills before it is mixed with ballast for preparing tunnel and shaft backfills. For complete elimination of the risk of contamination of living bacterial species the clay powder can be heated to about 400°C for one hour without altering its physical properties [12].

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