Microbial Processes Relevant for Long-Term Performance of Radioactive Waste Repositories in Clays

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1 Introduction

A number of investigations on occurrence and viability of microbes in compacted clays have been aimed at studying possible microbial effects on long-term performance of a deep geological repository (DGR) for high-level radioactive waste (HLW) and spent nuclear fuel (SF). Compacted clays are considered in current DGR designs either as a buffer material (compacted bentonite) or as a host rock (claystone). Accordingly, those investigations have been carried out in countries pursuing concepts of final HLW/SF disposal utilizing not only a claystone formation as a host rock for a DGR (Switzerland, France, Belgium[1]), but also a granitic formation (Sweden, Finland, Canada), for which the DGR performance largely relies on confinement properties of copper canisters amended by favourable chemical and mechanical properties of enveloping clay buffer /OND 01/, /NTB 02/, /AND 05/, /NWM 05/, /SKB 06/, /POS 07/. Therefore, a survey of investigations of microbes in compacted clays in the current analysis provides a compilation of issues relevant for the long-term performance of a DGR in granitic and claystone formations.

Estimates based on numerous experimental observations suggest that more than 20 % of prokaryotes on earth reside in the terrestrial and marine subsurface sediments below 200 m /WHI 98/. Albeit it is currently impossible to present reliable numbers concerning the deep subsurface biomass, which is mostly due to the lack of representative drill cores covering all subsurface environments /REI 11/, this estimate may hold true, as it still did not include microbes living below 400 m in the ocean crust. Recent research presented conclusive evidence that living microbes are present in very old (16 million up to 111 million years) sediments in an abundance of more than 10^6 cells per cm^{-3} as deep as 400 m up to 1.6 km below the seafloor /SCH 05/, /ROU 08/. For a comparison, aquatic environments contain 10^3 - 10^6 microbes per cm^{-3}, and a typical forest soil contains 10^6 microbes per gram of soil below the top one meter layer /WHI 98/.

Based on the abundance of phospholipid fatty acids indicative of living microbes, a 170-million-year-old sediment from the Opalinus Clay – considered as a candidate host rock for a DGR in Switzerland – has been estimated to contain 5×10^6 living microbes per g of dry claystone /MAU 07/, /STR 07/. Similarly, a 35-million-year-old sediment sampled at a depth of 224 m from the Boom Clay – considered as a candidate host

[1] Note that the Boom Clay considered in Belgium as a potential host rock for a DGR for HLW/SF is regarded as weak claystone in the specialist literature /DEH 04/.

rock for a DGR in Belgium – has been shown to contain living microbes /BOI 96/. The question is therefore not whether microbes will be present in a DGR in the post-closure phase, but rather whether they will exert a negative impact on the DGR performance in the long term and if so, to what extent?

A systematic evaluation of the relevance of microbial activity for the long-term performance of a DGR requires, as a prerequisite, the identification of (i) the safety-relevant processes, which can be accelerated (e.g., a conversion of smectite to illite) or aggravated (e.g., a change from uniform corrosion to pitting corrosion) by microbial activity, and (ii) the safety-relevant properties (e.g., swelling pressure) of the technical and geotechnical barriers and of the clay formation, which can be deteriorated as a result of microbial activity. This evaluation represents the primary purpose of the present analysis and is in turn an essential prerequisite for the quantitative estimation of the maximum possible effects of microbial processes in a DGR to be made in follow-up studies.

Such quantitative estimation should additionally take into account (iii) the changes in the repository's physical and geochemical conditions during the operation and post-closure phases, which can control microbial activity (e.g., increases in temperature, decreases in pore sizes and pore connectivity or increases in water content upon re-saturation of clay). This issue deserves a special attention, as limitations on water and space in a DGR in clay can strongly influence the size and activity of microbial populations /STR 07/, /STR 10a/, /STR 10b/. Its relevance for DGR design is demonstrated by the decision of the Canadian nuclear waste management organisation to abandon a mixture of 50 % bentonite and 50 % sand as the reference buffer sealing material and to select 100 % bentonite instead, in order to decrease the pore space available for microbial activity and to limit in this way the potential of microbially influenced corrosion of waste canister /NWM 05/. A further subject of essential importance for assessing long-term performance of a DGR in clays, quantification of which remains open for future investigative efforts, concerns (iv) the impact of microbial activity on radionuclide transport and radionuclide redox chemistry in clay environments.

In the following, an overview is given of the processes in the clay buffer, at the clay buffer–waste canister interface, at the clay buffer–host rock interface, and in the claystone within a DGR, which have been found so far to be initiated or influenced by the activity of microbes either indigenous to the repository's host rock or inoculated during the construction and operation of a repository. The order, in which these processes are

considered, is predetermined by the ability of microbes, responsible for the onset or a modification of a process, to compete for sources of energy required for maintaining the process. Microbes utilize redox processes as a general way to gain energy for metabolism and growth by directing the flow of electrons from the donor substrates to an intermediate, intracellular electron acceptor and from that to the terminal, extracellular electron acceptor. With respect to the limiting condition of energy accessibility, subsurface sediments can be divided into two broad categories of electron-donor limited and electron-acceptor limited ones /CHA 00/. Pristine sediments with the content of organic carbon – representing an essential source of electrons for microbial metabolism – being lower than that of electron acceptors (O_2, Fe(III), SO_4^{2-}, and CO_2) are regarded thereby as limited in electron donors.

Clays are generally characterized by organic carbon contents in the range of 0.1 - 5.0 % (e.g., ~0.6 % in Opalinus Clay /NTB 02/, up to 1.1 % in Callovian–Oxfordian argillite /GAU 04/, and 1 - 5 % in Boom Clay /BOI 96/, /BER 97/) on a dry weight basis. Since sulphate and carbonate are only present as accessory minerals and/or pore water solutes in clays, the latter values are to be compared to amounts of structural Fe(III), the then predominant electron acceptor in anoxic clays. Major Fe(III)-containing clay minerals – illites, smectites, and chlorites – constitute ~35 - 45 weight % in the aforementioned three clay formations /BER 97/, /NTB 02/, /GAU 04/. Considering that these clay minerals can contain up to ~23 % Fe(III) by weight /SEA 06/, /JAI 07b/, /DON 09/, at maximum ~10 % Fe(III) by weight can be expected in an undisturbed clay formation with actual value being very likely much lower.

Based on the ratio of ~4.7 between standard atomic weights of iron and carbon, the number of iron ions corresponding to this Fe(III) content is equivalent to the number of carbon atoms corresponding to the organic carbon content of ~2.2 weight %. However, four Fe(III) ions can be assumed to be reduced upon microbial metabolism of one carbon atom (see the summary of the specifications of the safety-relevant process “Microbial reduction of clay minerals”). Accordingly, clay formations considered currently as candidates to host a DGR for HLW/SF – Opalinus Clay, Callovian–Oxfordian argillite, and Boom Clay – contain Fe(III) in amounts sufficient to reduce carbon amounts equivalent to organic carbon content of only ~0.6 weight % at maximum and cannot be unambiguously classified as electron-donor limited systems. Therefore, both electron-donor limited setting and more complicated electron-acceptor limited setting will be dealt with in this overview.

In deep subsurface systems with limited supply of electron donors and under anaerobic conditions, which can be expected to establish in a DGR several years after its closure, the Fe(III)-reducing microbes outcompete sulphate-reducing and methane-producing microbes for electron donors and thus effectively inhibit sulphate reduction and methane production /LOV 87/, /HUR 97/, /CHA 00/. Therefore, the processes of microbial reduction and microbial dissolution of clay minerals related to the activity of Fe(III)-reducing microbes are dealt with first in this overview. Similarly, sulphate-reducing microbes producing sulphide[2], which is corrosive to the metal canisters, maintain dissolved concentrations of electron donors at concentrations too low for methanogenic microbes to produce methane /LOV 87/, /HUR 97/, /CHA 00/. The methane production will hence only occur either after the sulphate-reducing microbes have consumed sulphate or in local environments not conducive to sulphate-reducing microbes. Accordingly, the process of microbially influenced corrosion is discussed in advance of the process of microbial gas production, which is not limited to – but strongly influenced and contributed by – methanogenic microbes. Since biofilm formation appears often to be the precursor to microbially influenced corrosion of metal surfaces, consideration of the biofilm formation precedes that of the microbially influenced corrosion.

The consideration of these five major microbial processes in clays is followed by a discussion of the case of limited availability of electron-acceptors in a DGR utilizing a clay formation as the host rock. Such subsurface setting can occur in particular as a result of contamination during industrial or mining activities and be characterized by the reverse dominance order of microbial reduction processes with, e.g., methanogenesis being the predominant microbial reduction process near the contaminant source. The situation is in this case further complicated by the often observed significant concomitant activities of microbes responsible for different competing reduction reactions. Excavation of a DGR, placement of radioactive waste, as well as backfilling and sealing of the DGR will most probably change the pristine characteristics of the host formation in the disturbed part of the containment-providing rock zone[3] by adding either electron-

[2] Sulphide can be present as H_2S, HS^- or S^{2-} in aqueous solution depending on pH value.

[3] from German: einschlusswirksamer Gebirgsbereich

donors or electron-acceptors to the system[4]. As possibly the most complicated – for quantification of impact of microbial processes when assessing long-term performance of a DGR – outcome of this kind of disturbance, an originally electron-donor limited system may become an electron-acceptor limited one and vice versa. The last but one section of this overview is therefore aimed at extending the consideration of subsurface systems limited in electron donors in the preceding sections to those limited in electron acceptors. In the last section, the effects of the considered microbial processes on long-term performance of radioactive waste repositories in clays are summarized and a research agenda for future work in this field is proposed.

[4] This overview does not consider the activity of nitrate-reducing microbes, the influence of which for a DGR for HLW/SF in subsurface clays should be assessed based on the on-going inventory studies for vitrified waste. For the case that nitrate inventories in vitrified waste should be non-negligible as well as for an analysis of the long-term performance of a DGR for medium-level radioactive waste, which can contain large quantities of nitrate, a consideration of the process of microbial nitrate reduction and its interplay with the other microbial processes need to be carried out.

2 Microbial reduction of clay minerals

It is now accepted that microbial metabolism – and not abiotic mechanisms – primarily controls iron redox chemistry in most environments /WEB 06/. According to a recent review /DON 09/, a wide variety of microbes has been found to reduce structural Fe(III) in iron-containing clay minerals, such as montmorillonite, nontronite, illite, chlorite, palygorskite or biotite, to maintain their metabolism. To prevent cell death by suffocation in the absence of dissolved electron acceptors /REI 11/, these usually facultative or obligatory anaerobic microbes use insoluble Fe(III) as a terminal electron acceptor /DON 09/. Three primary mechanisms have been proposed to explain the transfer of electrons to extracellular Fe(III) incorporated in the mineral structure /WEB 06/, /DON 09/: (a) the direct contact between the microbe and the clay mineral, (b) the production of endogenous/use of exogenous soluble electron shuttles, and (c) the production of chelating ligands to facilitate the mineral dissolution providing soluble Fe(III).

(a) Two modes of the direct microbe–mineral interaction providing electron transfer are currently known /WEB 06/. One mode requires a very close microbe approach to the mineral surface (Fig. 2.1 A), so that electron transfer can occur via metalloproteins associated with the outer cell membrane. In another mode, which has been demonstrated recently for several microbial species (metal-reducing, oxygenic photosynthetic, and thermophilic fermentative ones), microbes form extracellular appendages – called bacterial nanowires owing to their electrical conductivity (Fig. 2.1 B) – in response to electron-acceptor (O_2) limitation in order to transport electrons to solid-phase Fe(III) /GOR 06/, /ELN 10/.

    As a result of such electron transport, poorly crystalline ferrihydrite ($Fe_2O_3 \cdot n\ H_2O$) has been observed to reductively transform into nanocrystalline magnetite (Fe_3O_4) along bacterial nanowires, which are composed of individual filaments and characterized by a diameter of 50 - 200 nm and the length of several tens of microns /GOR 06/. These structures can, on the one hand, make reactive surfaces confined in submicron pores accessible to microbes and, on the other hand, provide a mean to maintain microbial metabolism in environments where microbes are sessile.

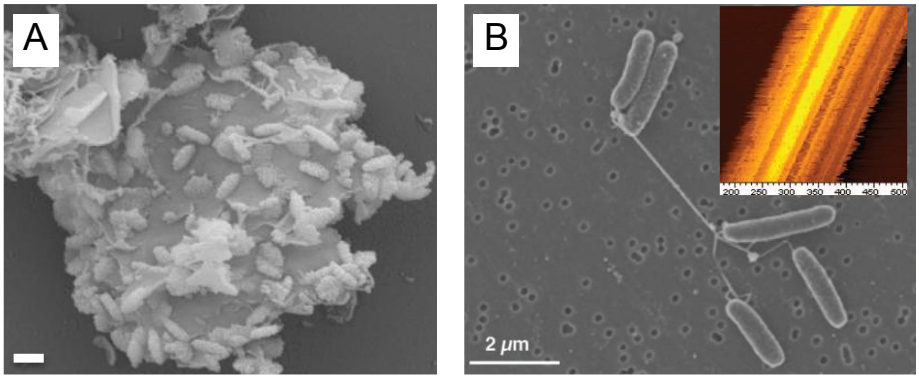

Fig. 2.1 Scanning electron microscopy micrographs of Fe(III)-reducing microbes on the surface of the Fe(III)-rich clay mineral nontronite (scale bar corresponds to 2 µm) (A) /JAI 07a/; and of Fe(III)-reducing microbes connected with each other and with the substrate by bacterial nanowires (B) composed of bundles of individual filaments as seen at higher magnification (inset, scale in nanometres) /GOR 06/

(b) In an alternative active mechanism of electron transfer, which alleviates the need for the direct microbe–mineral contact, microbes reduce a soluble electron shuttle that diffuses to the mineral surface and donates electrons to the structural Fe(III). Redox-reactive organic compounds common in sediments, such as humic acids, have been identified as exogenous electron shuttles. In humic acids, quinone moieties accept electrons, and the resultant hydroquinones donate electrons to Fe(III) acting as the terminal electron acceptor /LOV 96/. Model compounds containing quinones have been confirmed to increase the extent of clay mineral reduction – i.e., the amount of structural Fe(III) ions reduced within the clay mineral matrix – by a factor of 2 - 4 as well as the rate of this reaction /LOV 98/, /JAI 07b/. These increases have been suggested to (partially) result from diffusion of electron shuttles into nanometre-sized pores, which contribute a considerable fraction in clay minerals and would be otherwise not or only hardly accessible to microbes.

The absence of exogenous electron shuttles can be overcome by microbes through the production of endogenous electron shuttles. The endogenous flavin mononucleotide and riboflavin (also known as vitamin B_2) have been shown to transfer two electrons per molecule with 1 mmol ml^{-1} of flavins reductively producing 2 mmol ml^{-1} of Fe(II) from insoluble Fe(III) oxide /VON 08/. These flavins were actively secreted by the studied facultative anaerobes equally efficiently under both aerobic and anaerobic conditions and were as effective electron shuttles as exoge-

nous quinone-containing compounds. In another experiment, ~7 × 10^8 ml^{-1} of microbes have been demonstrated to produce 1.1 µmol ml^{-1} of Fe(II) from poorly soluble Fe(III) (hydro)oxide within three days due to an anaerobic production of non-quinone electron shuttles /LIE 05/, which have been later inferred to be flavins /MAR 08/ and which allowed to transfer electrons to metals separated by more than 50 µm from microbes.

Widespread observed production of flavins under aerobic conditions could appear energetically wasteful, since electron shuttles are not needed when oxygen serves as the terminal electron acceptor. However, it has been argued that microbes living in interfacial environments, such as redox fronts, may benefit from the flavins secreted during aerobic growth, as a quick change from oxic to anoxic conditions makes necessary an as quick change to anaerobic respiration of solid-state Fe(III) /VON 08/. Furthermore, at the microbe-mineral interface, the metabolic expense of secreting flavins may be an energetically favourable process, as they could be used in multiple rounds of extracellular electron shuttling. This recycling advantage may become even more important in microbial biofilms /WEB 06/, formation and impact of which are dealt with as the safety-relevant process “Biofilm formation”. Last but not least, the biosynthesis of flavins has been estimated to cost less than 0.1 % of the microbe’s budget of adenosine triphosphate molecules at the nutrient-rich conditions, at which an accumulation of 0.25 nmol ml^{-1} of riboflavin increased the rate of electron transfer by 70 % /MAR 08/.

(c) The third active mechanism, by which microbes can access the solid-phase Fe(III) for electron transfer, involves the use of exogenous chelating compounds or the production of endogenous ones to solubilize Fe(III). Along with the solubilisation of Fe(III) from the clay structure, which is discussed in detail for the safety-relevant process “Microbial dissolution of clay minerals”, exogenous organic compounds chelating Fe(III) have been shown to lead to an up to twofold increase in the extent of microbial clay reduction /KOS 99a/.

The extent of clay mineral reduction determines the severity and reversibility of changes in clay mineral properties /RIB 09/. A microbial smectite reduction limited to a production of ~1 mmol structural Fe(II) ions per g clay leads to an increase of negative charge of smectite layers by ~1 mmol elementary charges per g clay accompanied by a minor adjustment of the layer structure, which have been found to be fully reversible

upon re-oxidation of the microbially reduced samples /LEE 06a/, /RIB 09/. The latter observation confirms that microbial reduction of smectite proceeded by a solid-state transformation, which can follow either of the first two mechanisms of electron transfer introduced above. Furthermore, the presence of distinct domains of structural Fe(II) and Fe(III) in microbially reduced smectite /KUK 06/, /RIB 09/ has been interpreted as an evidence for Fe(III) reduction advancing from the edges of smectite layers toward their interior (compare with Fig. 2.2) /RIB 09/. Accordingly, the smectite edges have been concluded to represent the point of contact between the clay mineral and the microbe's cell membrane or electron shuttles.

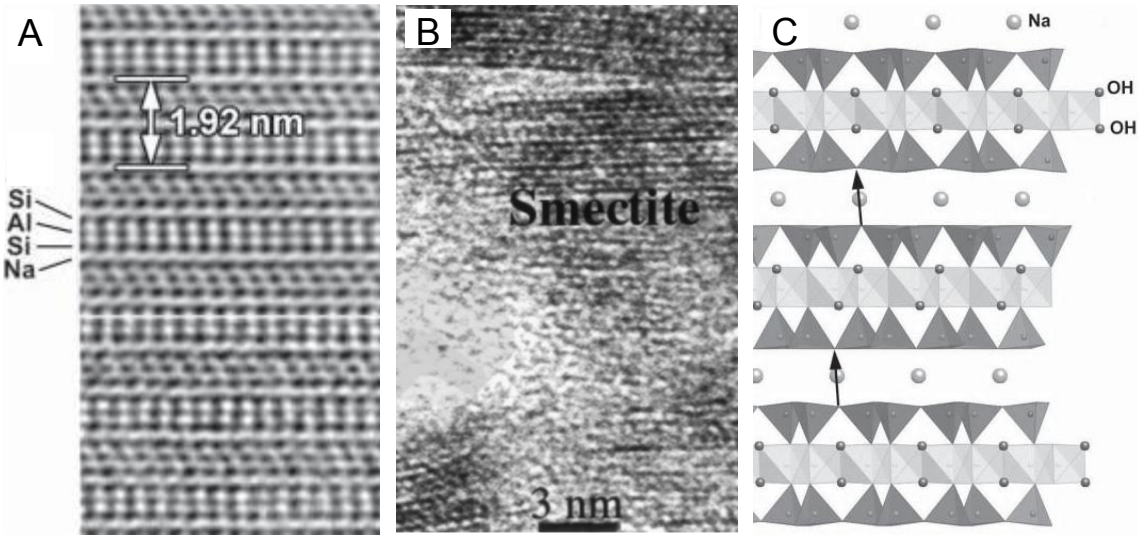

Fig. 2.2 High-resolution transmission electron microscope images of a paragonite (A) /KOG 07/ and a smectite (B) /BAN 99/ showing atomic structure of clay mineral layers. It can be seen from (A) and (C) /KOG 07/ that a single layer of these clay minerals consists of two sheets of tetrahedrally coordinated Si sandwiching a sheet of octahedrally coordinated Al, and the adjacent interlayer space is populated with – in this case dehydrated sodium – cations adsorbed at the basal surfaces of mineral layers (the rightmost OH^- groups mark the edge surface of the layers). In clay minerals, Fe(III) occurs as a substitution for Si(IV) or Al(III) in the mineral layer structure. Regard that clay mineral layers typically stack to packets of up to a few tens of mineral layers with only their edge surfaces (and the both lateral surfaces of the stack) remaining exposed to the pore solution (see (B) and, in the next section, Fig. 3.1 A)

The same conclusion has been made independently following the discovery of 10^8 presumably mostly iron-reducing microbes per cm^3 of 0.1 - 0.4-million-years-old ice at the bottom of the 3-km-thick Greenland icecap /TUN 06/. These microbes metabolized in the direct contact with micrometre-scale, predominantly smectite grains dispersed at a concentration of ~0.004 g/g of ice, and the number of attached microbes per grain was proportional to grain perimeter rather than to area, which implies that Fe(III) was accessed at grain edges rather than at basal planes (compare with Fig. 2.2). Differently from laboratory studies of microbial Fe(III) reduction, which are usually limited to a few weeks, the latter study provided evidence that this process has allowed for a survival of a thriving population of microbes (~10^{11} per g of dry clay) for as long as 0.1 - 0.4 million years. The authors have argued that microbial reduction of structural Fe(III) ions at grain edges, which exhausts the inventory of terminal electron acceptors accessible by microbes through a direct contact, should be accompanied by a concentration-gradient-driven Fe(II)–Fe(III) electron hopping into the interior of clay grains, which can at least partially recover Fe(III) concentration at the edge surface. The occurrence of electron hopping between adsorbed Fe(II) and structural Fe(III) in smectite has been confirmed in a recent experimental study /SCH 11/.

An increase of negative layer charge as a result of a microbial smectite reduction proceeding by a solid-state transformation and limited to a production of ~2 mmol structural Fe(II) ions per g clay is also accompanied by a decrease of swelling pressure (**safety-relevant property**) by ~40 %, an increase of cation exchange capacity (**safety-relevant property**) by ~20 - 30 %, and a decrease of specific surface area (**safety-relevant property**) by ~30 - 50 % /KOS 99b/, /STU 06/. These property changes were largely, but not completely, reversed upon re-oxidation and deviated by, respectively, 0 - 10 %, 10 - 20 %, and 10 % from the values for the unaltered smectite. It has been noted that cation exchange capacity changes directly, although not necessarily linearly, proportional with the extent of clay mineral reduction, which has been attributed to ancillary reactions, such as structural dehydroxylation. As a further – presumably reversible upon re-oxidation – consequence, microbial Fe(III) reduction significantly improves anion sorption capacity[5] (**safety-relevant property**) of clay minerals, as they become able to reduce and effectively immobilize highly mobile Tc(VII) existing as pertechnetate (TcO_4^-) anion under aerobic conditions /JAI 09/, /PER 09/.

[5] Note that the sorption of metal ions on mineral surfaces involves adsorption, surface precipitation, and co-precipitation phenomena /CHA 92/.

The whole body of the experimental evidence indicates that the effects of microbial reduction of Fe(III) in clay minerals are very similar to those of chemical reduction, if the extents of the reduction are similar /STU 06/, /RIB 09/. It has been shown that a chemical solid-state reduction of smectite exceeding ~3 mmol Fe(II) per g clay results in very strong and largely irreversible – except for dehydroxylation and the Fe oxidation state – changes in clay structure and properties /FIA 02/, /RIB 09/. These changes include, amongst others, a decrease of cation exchange capacity (**safety-relevant property**) because of a permanent cation retention (cation fixation) observed for Na^+, K^+, Ca^{2+}, Cu^{2+}, and Zn^{2+} ions as a result of the progressive interlayer collapse[6] /KHA 91/, which also decreases swelling pressure (**safety-relevant property**). However, although such strong changes have been predicted for microbially reduced smectites, neither they nor a microbial reduction exceeding ~2 - 3 mmol Fe(II) per g clay have been observed so far /STU 06/, /DON 09/.

One probable reason for this lacking evidence is the increasing susceptibility of clay mineral structure to dissolution as a result of microbial reduction proceeding by solid-state transformation /KUK 06/, /STU 06/, so that at least some of the Fe(II) is released from the clay structure and inhibits further microbial reduction of solid-state Fe(III) /DON 09/ as discussed below for the safety-relevant process “Microbial dissolution of clay minerals”. Another reason may be a decreasing accessibility of Fe(III) buried within the packets of clay mineral layers (Fig. 2.2 A) upon an increasing extent of solid-state transformation. The maximum value of layer charge compatible with the ability of clay minerals to swell is generally considered to be ~1.3 mmol elementary charges per g clay /SEA 06/. As soon as clay minerals reach such a layer charge in the course of microbial reduction of structural Fe(III), the progressive collapse of interlayer spaces between single mineral layers would render remaining structural Fe(III) inaccessible for a rather fast and effective electron transfer across the basal surfaces of the mineral layers. Indeed, the results of the latter study suggest that microbial reduction of Fe(III)-containing illites was occurring as long as exogenous quinone-containing compounds were able to enter the interlayer spaces in order to deliver electrons to structural Fe(III) but ceased upon an increase of layer charge to the above limiting value.

[6] Note that the increase of negative layer charge, which initially leads to the corresponding increase of cation exchange capacity, can eventually result in a decrease of cation exchange capacity due to the interlayer collapse, which displaces water from interlayer spaces and makes trapped interlayer cations hardly available for cation exchange with pore solution species.

2.1 Evaluation of the safety relevance of the process "Microbial reduction of clay minerals"

It had been assumed until early 1990s that the reduction of Fe(III), which is an important process in a diversity of anoxic environments, is the result of abiotic reactions at a low redox potential /LOV 97/. However, direct evaluations of this hypothesis have proved that in deep sedimentary environments with insignificant sulphide production, Fe(III)-reducing microbes primarily control Fe(III) reduction /LOV 97/, /WEB 06/. They still make a considerable contribution to Fe(III) reduction in sediments where microbial reduction of sulphate to sulphide, which can reduce Fe(III) in an abiotic reaction as discussed below for the safety-relevant process "Microbially influenced corrosion and activity of sulphate-reducing bacteria", dominates the microbial activity /CAN 93/.

Importantly, the ability to reduce solid-state Fe(III) is not restricted to microbes using exclusively Fe(III) as terminal electron acceptor but is also possessed by sulphate-reducing or methane-producing microbes (see discussion for the safety-relevant processes "Microbially influenced corrosion" and "Microbial gas production" for more details). Therefore, in the presence of electron donors in a DGR in amounts sufficient for activity of either of the three competing groups of microbes – the issue discussed in detail in the section on electron-acceptor limited subsurface settings below, microbial reduction of Fe(III) should be considered a relevant process for DGR safety.

Even if this process may be extremely latent in the indigenous host rock due to a limited supply of electron donors or water or because of a high compaction of host rock material during the post-depositional history, the excavation of a DGR will inevitably – though most probably only partially and temporarily – lift these limitations. Backfilling of DGR excavations during its closure will not lead to a complete restoration of pristine host rock conditions – at least within a sizeable period of time – and will leave microenvironments conducive to microbes in a DGR /DEC 04/, /KUR 04/, /AER 09a/, /AER 09b/, /STR 11/, /WER 11a/. The ability of microbes to use sophisticated systems – either nanowires or shuttles – to transfer electrons to clay Fe(III) will additionally ease the limitation of local availability of terminal electron acceptors imposed on activity of microbial population.

Furthermore, the second most hyperthermophilic microbe species[7] discovered so far – strain 121, which has been shown to double its cell number after 24 hours at 121 °C, uses exclusively Fe(III) as terminal electron acceptor /KAS 04/. In fact, almost all hyperthermophilic microbes investigated so far have been found to reduce Fe(III) /LOV 04/. These microbes have been isolated from deep subsurface sediments, hydrothermal groundwaters or vents and are characterized by optimum growth temperatures in the range of ~80 - 121 °C, which covers the maximum temperature of ~100 °C at the interface between waste canisters and clay buffer prescribed in the concepts of final disposal of radioactive waste.

Moreover, these microbial species have the ability to thrive on molecular hydrogen (H_2) as the only electron donor. Importantly, this energy source for activity of Fe(III)-reducing microbes may be supplied in the vicinity of waste canisters not only by corrosion reactions at the canister–water interface (see discussion for the safety-relevant process "Microbially influenced corrosion and activity of sulphate-reducing bacteria") or by water radiolysis but also by microbially mediated generation of H_2 from minerals as revealed recently /REI 11/ (see discussion for the safety-relevant process "Microbial gas production"). These considerations and the ability of Fe(III)-reducing microbes to potentially directly influence four safety-relevant properties of the clay buffer and the clay host rock point up the need for accounting for their possible impact when assessing long-term performance of a clay-utilizing DGR.

2.2 Summary of the specifications of the process "Microbial reduction of clay minerals"

Safety-relevant properties of clay influenced:

(i) swelling pressure (negatively influenced),
(ii) specific surface area (negatively influenced),
(iii) cation exchange capacity (positively influenced unless the increase of cation exchange capacity is large enough to trigger cation fixation, which exerts strongly negative influence on this property),
(iv) anion sorption capacity (positively influenced).

[7] The most hyperthermophilic microbe species is a methanogen thriving at 122 °C and 20 MPa (see discussion for the safety-relevant process "Microbial gas production" for further details).

Barriers with safety-relevant containment function affected:

(i) clay buffer,
(ii) claystone.

Mechanisms:

(i) solid-state transformation of the clay mineral structure upon a direct contact with microbial cells,
(ii) interactions with soluble shuttles delivering electrons produced during microbial metabolism.

Reactions involved[8] (a relevant selection):

Reduction of aqueous Fe(III) coupled to hydrogen oxidation /THA 77/ – the stoichiometry of this reaction applies also to structural Fe(III) in clays /SHE 03/,

$$2\ Fe^{3+} + H_2 \rightarrow 2\ Fe^{2+} + 2\ H^+, \qquad (2.1)$$

with $\Delta G°'$ = −228 kJ/reaction.

Reduction of Fe(III) hydroxide coupled to oxidation of acetate /MCM 95/

$$8\ Fe(OH)_3 + CH_3COOH + 16\ H^+ \rightarrow 8\ Fe^{2+} + 2\ CO_2 + 22\ H_2O, \qquad (2.2)$$

with $\Delta G°'$ = −51 kJ/reaction,

or lactate /KOS 02/

$$8\ Fe(OH)_3 + CH_3CHOHCOO^- \rightarrow$$
$$4\ Fe_3O_4 + CH_3COO^- + HCO_3^- + H^+ + 18\ H_2O, \qquad (2.3)$$

with $\Delta G°'$ = −410 kJ/reaction.

Reduction of Fe(III) in smectite coupled to lactate oxidation /KOS 02/

$$1.47\ [Na_{0.81}(Si_{7.3}Al_{0.7})(Al_{1.06}Fe(III)_{2.73}Mg_{0.26})O_{20}(OH)_4] +$$
$$CH_3CHOHCOO^- + 2\ H_2O \rightarrow$$

[8] Free energy increments $\Delta G°'$ are given according to /THA 77/ and /KOS 02/ for reactions under standard conditions – i.e. 25 °C, 0.1 MPa, water activity of 1, solute activities of 1 mol kg^{-1} – except that physiological pH value of 7 is taken instead of the standard activity of 1 mol kg^{-1} for H^+, which corresponds to pH value of 0.

$$1.47\ [Na_{0.81}(Si_{7.3}Al_{0.7})(Al_{1.06}Fe(II)_{2.73}Mg_{0.26})O_{20}(OH)_4]^{-2.73} + CH_3COO^- + HCO_3^- + 5\ H^+ \quad (2.4)$$

with $\Delta G°'$ = −436 kJ/reaction.

3 Microbial dissolution of clay minerals

3.1 Reductive dissolution

The processes of microbial dissolution and reduction of clay minerals are intimately related to each other as they both are triggered by the microbial demand for Fe(III) as terminal electron acceptor. A recent study has suggested that upon a solid-state microbial reduction of ~1.2 mmol Fe(III) per g clay, smectite structure destabilizes and a release of structural Fe(II) becomes possible /JAI 08/. Accordingly, a progressive reduction of up to ~3 mmol of structural Fe(III) per g clay within several days resulted in a progressively increasing release of up to ~1.5 mmol of Fe(II) per g clay, which partitioned to surface complexation sites followed by ion-exchangeable sites and, upon the saturation of these sorption sites on the smectite surfaces, into the aqueous solution. In an earlier study utilizing the same microbial species but different experimental conditions and smectite types, ~0.04 mmol of structural Fe(II) per g clay were released upon a reduction of ~0.57 mmol of structural Fe(III) per g clay within 50 days /KOS 99a/.

The increasing release of Fe(II) and its sorption on clay mineral and microbe surfaces, however, have been reported to increasingly inhibit and to eventually stop the reduction of structural Fe(III) in clay minerals and, consequently, their dissolution /JAI 07b/. The latter study has suggested that this adsorbed Fe(II) may (partially) block the electron transfer chain from microbe to mineral surfaces. In an alternative explanation, formation of an Fe(III) oxide on the clay surface as a result of an interfacial electron transfer between sorbed Fe(II) and structural Fe(III), which has been observed recently, would allow microbes to start a direct reduction of the secondary Fe(III) oxide rather than the clay mineral /SCH 11/.

In the presence of sufficient dissolved potassium, microbial Fe(III) reduction and clay dissolution result in the irreversible conversion of smectite to illite (with the latter being poorer in Fe(III) than the former). Without microbial activity, this most important diagenetic clay reaction occurs in mudstones and shales over a temperature range of 50 - 180 °C and geological times of 0.5 - 300 million years /POL 93/, but can be substantially accelerated to be completed within 4 - 5 months at 300 - 350 °C and 100 MPa /KIM 04/. Due to microbial activity, however, a strong acceleration of this reaction takes place, which results in the reduction of ~1.2 mmol Fe(III) per g smectite and the formation of illite in as little as 14 days at as low temperature and pressure as of 25 °C

and 0.1 MPa /KIM 04/. It has been concluded in that study that the illite formation proceeded by a continuous nucleation and growth mechanism, implying the reductive dissolution of smectite and simultaneous precipitation of illite (Fig. 3.1 A).

A wide range of thermophilic and hyperthermophilic[9] microbes from marine and freshwater environments has been found to reduce up to ~0.5 mmol structural Fe(III) per g smectite and to partially release dissolved Fe(II) and Fe(III) within three days at temperatures varying from 55 to 115 °C /KAS 08/. The limited duration of the experiment as well as the preferential adsorption of magnesium instead of potassium in the interlayer spaces of microbially reduced smectite rendered, however, the observed increase of its layer charge (by ~33 %) insufficient to form an illite-like structure.

A recent study of Middle Cambrian mudstones from the western United States has presented a strong evidence of microbial smectite dissolution accompanied by precipitation of illite, which occurred at temperatures in the range from 26 to 69 °C during early diagenesis /VOR 09/.

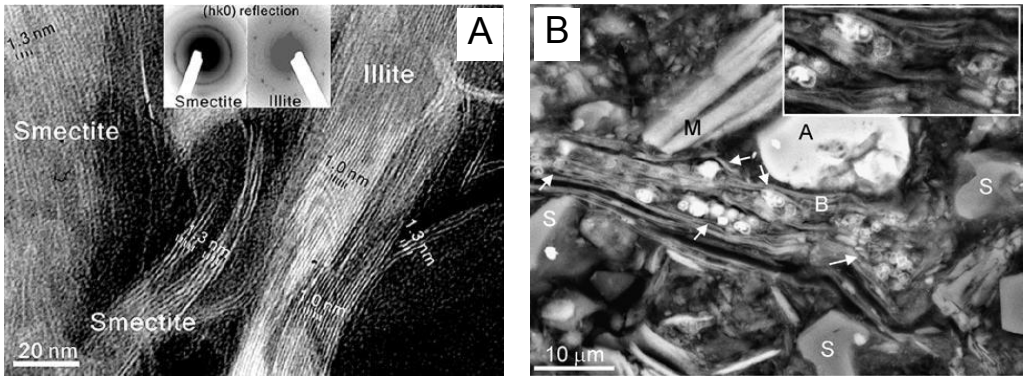

Fig. 3.1 Transmission electron microscopy micrograph of the microbially reduced smectite sample with a ~40-nm-thick packet of 1.0-nm illite layers embedded in the matrix of 1.3-nm smectite layers (A) /KIM 04/. Scanning electron microscopy micrograph of iron-rich sulphides (arrowed) arranged between the exfoliation planes of biotite (denoted by B) grains (B). The coccoid morphology of the iron-rich phases can be clearly seen at higher magnification (inset with a width of 20 µm) /SAN 09/

[9] Mesophilic, thermophilic, and hyperthermophilic microbes are characterized by a preferential grows around 30, 60, and 90 °C, respectively /SPA 00/.

Similarly, Lower Miocene mudstones and dolomites from Madrid Basin in Spain provide a conclusive mineralogical record of microbial reduction of Fe(III) and release of Fe(II) from biotite and chlorite that were depleted of Fe by up to 24 and 40 weight %, respectively /SAN 09/. Microbial dissolution of biotite in these sediments was accompanied by microbial sulphide production and precipitation of iron sulphides, which were either formed along the exfoliation planes of biotite grains (Fig. 3.1 B) or replaced biotite crystallites inheriting thereby structural habits of the latter. Furthermore, a field study carried out with millimetre-sized plates of Fe(III)-bearing muscovite placed in an Antarctic Dry Valley stream has documented a Fe depletion of muscovite structure and an alignment of microbes along the edges of muscovite layers /MAU 02/.

Importantly, illite neo-formed from dissolution of smectites, as discussed above, but also from kaolinites, micas, and K-feldspar in bentonites, sandstones, mudstones, shales or altered volcanic rocks, precipitates directly from solution in pore space /NAD 02/, /VOR 09/. Moreover, microbial reduction and extraction of structural Fe in clay minerals leads also to a concomitant release and precipitation of silica in pore space /VOR 09/, /DON 09/. The illite and silica particles neo-formed in such a way are submicrometre-sized and can readily fill the void spaces of a comparable or smaller size, which control the permeability in mudstones /NAD 02/, claystones /BOI 96/, /MAU 07/ or clay buffers compacted to a DGR-relevant dry density of ~1.6 g cm^{-3} /STR 10b/. It is argued that relatively minor amounts of neo-formed illite, less than 5 %, within mudstones can greatly reduce permeability by blocking the effective pore network, and thereby render them prone to overpressure /NAD 02/. Similarly, microbial dissolution of clay minerals is argued to be responsible for much of the early silica cementation occurring within shales and adjacent rocks /VOR 09/, which can contribute to the overpressure development /NAD 02/.

It can be concluded from the above discussion that microbial dissolution of clay minerals can result in a decrease of porosity (**safety-relevant property**) and a decrease of permeability (**safety-relevant property**). These decreases are accompanied by an increase of fluid pressure (**safety-relevant property**) up to a development of overpressure that can eventually lead to the temporal loss of clay plasticity (**safety-relevant property**) accompanied by formation of fractures /ORT 02/, /DEH 04/, and to fluid discharge (loss of the **safety-relevant containment function** of the clay) to relieve the excess pressure. From available geological records, changes in the former two properties can be regarded to be irreversible, whereas the overpressure release obviously

allows the recovery of the pre-dissolution state of the latter two properties rendering them reversible. Following the consideration of changes in safety-relevant properties as a result of microbial Fe(III) reduction of clay minerals in the preceding section, it can be concluded that microbial dissolution of swelling smectites and neo-formation of non-swelling illites results in irreversible decreases of smectites' swelling pressure (**safety-relevant property**), cation exchange capacity (**safety-relevant property**), and specific surface area (**safety-relevant property**). Furthermore, decrease of content of Fe(II) incorporated in the structure of clay mineral layers as a result of their dissolution should be considered as detrimental to anion sorption capacity (**safety-relevant property**) of clay.

3.2 Non-reductive dissolution

Besides increasing the extent of microbial clay reduction as discussed for the safety-relevant process "Microbial reduction of clay minerals", exogenous organic compounds (nitrilotriacetic acid, oxalate, citrate, malate) have been shown to facilitate microbial dissolution of smectite /KOS 99a/. These low-molecular-weight organic acids or their conjugates, which are weak Fe(III)-chelators, have been, however, not able to solubilize Fe(III) from smectite in the absence of microbes. Similarly, dihydroxybenzoic acid, a weak Fe(III)-chelator as well, has been found ineffective in solubilizing Fe(III) from microbe-free illite suspensions /PAG 84/. On the contrary, a significant release of structural Fe in illite and accumulation in microbes has been observed in the latter study when low-molecular-weight compounds with a very high and specific affinity for Fe(III) – siderophores (Fig. 3.2), of which almost 500 are currently known /BOU 02/, – were produced by microbes.

For a smectite, a release of up to ~6 µmol Fe(III) g^{-1} within six days has been observed as a result of a strong adsorption (up to ~0.6 mmol g^{-1} corresponding to ~70 % of cation exchange capacity of the smectite) of siderophores and Fe(III)-siderophore complexes, whereas Fe(III)-dissolution effect and adsorption of acetohydroxamic acid were negligible /SIE 04/, /HAA 08/. Siderophore-induced dissolution of Fe(III) from clay mineral palygorskite is comparable to that from the smectite despite an order of magnitude lower siderophore adsorption on the former /SHI 10/.

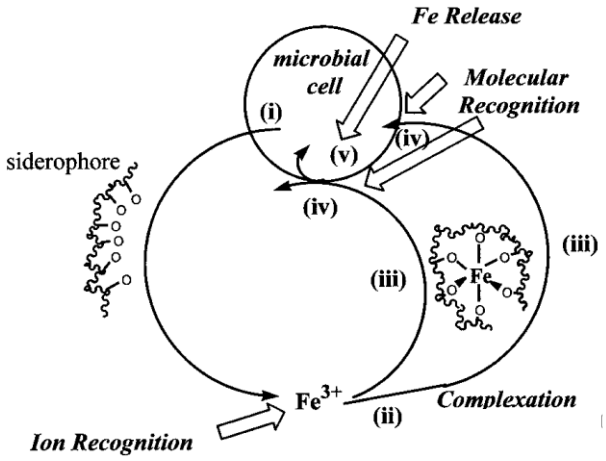

Fig. 3.2 Schematic representation of siderophore-mediated acquisition of Fe(III) by microbial cells: (i) siderophore synthesis and release by the cell; (ii) Fe(III) ion recognition and complexation; (iii) diffusion to the cell surface, (iv) siderophore complex molecular recognition by cell surface receptor; (v) iron release to the cell interior /BOU 02/

Furthermore, oxalate characterized by a more than 20 orders of magnitude lower affinity to Fe(III) than a siderophore has been observed to be up to 20 times less effective in dissolving goethite /CHE 03/. Yet, oxalate adsorption on goethite was strongly preferred over that of the siderophore, possibly due to by an order of magnitude larger size of the latter. Moreover, in the presence of oxalate, the dissolution of goethite by the siderophore increased up to fourfold. Based on these observations, it has been concluded that the synergistic dissolution effect of oxalate and siderophore comes about by continual Fe-oxalate detachment from the goethite surface followed by a rapid removal of the detached Fe by the dissolved siderophore making the detached oxalate available for repeated reaction with the goethite surface.

Similarly, siderophores have been shown to increase the extent of dissolution of Fe(III)-rich chain silicate hornblende by a factor of 2 - 4 as compared to acetic, oxalic or citric acids in line with the 19 - 27 orders of magnitude higher affinity of the siderophore to Fe(III) /BRA 04/. In the same synergistic effect as discussed above for goethite, acetate more than doubles dissolution of Fe(III) by a siderophore from a synthesized hornblende glass /BUS 07/. An observation of the increased dissolution of Fe from a Fe(III)-rich smectite by microbes that produced high-molecular-weight extracellular polysaccharides has led to a proposal that these anionic compounds may be functionally similar to Fe-chelators and be responsible for dissolving Fe from the clay mineral structure

/KAS 08/. However, experiments with the hornblende glass provided a strong evidence that Fe dissolution was not due to extracellular polysaccharides but rather acetate they contained /BUS 07/.

A non-linear dependence of the Fe release rate from hornblende on siderophore concentration has been ascribed to a formation of Fe(III)–siderophore complexes at the surface of hornblende prior to the Fe release /KAL 00/. This conclusion is supported by atomic force microscopy data suggesting a bidentate coordination of a siderophore molecule to the surface of goethite (α-FeOOH) /KEN 03/. The formation of a Fe(III)-siderophore surface complex is considered to weaken the bonds between Fe(III) and the mineral lattice and to result in a solubilisation of chelated Fe(III) – and not of reduced Fe(II) – in agreement with the observation of dissolved Fe(III) concentrations of up to 8 µmol l^{-1} at pH of 7.4 /BRA 04/ as compared to that of ~10^{-4} µmol l^{-1} in the absence of chelating compounds /BOU 02/. This observation of non-reductive Fe(III) dissolution is also consistent with the by some 20 orders of magnitude higher stability of Fe(III)–siderophore complexes as compared to Fe(II)–siderophore complexes, which are observed to rapidly dissociate /BOU 02/.

The same two-step Fe(III) chelation–solubilisation pathway has been suggested for the respiration of solid Fe(III) oxides by strictly anaerobic microbes following detection of soluble organic-Fe(III) prior to detection of Fe(II) /NEV 02/. Differently than aerobic and facultative anaerobic microbes, however, strictly anaerobic microbes are not known to produce siderophores. Although the identity of the microbially-produced chelator(s) dissolving Fe(III) prior to its reduction within microbial cells has not been determined in the latter study, a further study has confirmed that anaerobic respiration of solid-state Fe(III) by microbes proceeds without siderophore-synthesis /FEN 10/. In consistency with the mechanism of Fe solubilisation by oxalate /CHE 03/ discussed above, it can be therefore concluded that the non-reductive Fe(III) solubilisation is characteristic to non-siderophore chelators as well.

3.3 Evaluation of the safety relevance of the process "Microbial dissolution of clay minerals"

The reductive dissolution of clay due to microbial activity has been proved to have played an important role during the early stages of formation of sediment deposits including clays and to be able to induce severe changes to clay structure and properties.

The importance of this process for long-term performance of a clay-utilizing DGR can possibly be the best exemplified by considering that the concern about the transformation of smectite to illite has led to imposing the requirement on the DGR design in Belgium that the Boom Clay be not subjected to temperatures above ~100 °C /OND 01/, /OND 04/.

Although to the author's knowledge no direct evidence on the occurrence of this process in deep clay formations has been presented since the discovery of ability of microbes to gain energy from Fe(III) reduction two decades ago, clays generally fulfil the three necessary conditions for activity of Fe(III)-reducing microbes /LOV 90/: the presence of (1) such microbes, of (2) Fe(III), and of (3) organic matter. The general applicability of the first condition for undisturbed clay can be conservatively assumed from the observation that Fe(III)-reducing microbes are present and active in the industrial, ~35-million-years-old clays mined in Georgia, USA /SHE 05/ and from the lacking compelling evidence for their absence in the other clay formations investigated so far. A recent in situ experiment in the Opalinus Clay formation has additionally demonstrated that even if the undisturbed clay might have been devoid of Fe(III)-reducing microbes, they would most probably be introduced there by human activity during DGR construction and operation /STR 11/, /WER 11a/.

Evidences for the occurrence of this process in deep – though not necessarily clay – subsurface environment are numerous. In pristine aquifers, high groundwater concentrations of Fe(II), which is produced as a result of microbial reduction of solid-state Fe(III), is one of the most prevalent problems concerning potable water quality worldwide /LOV 04/. Furthermore, microbial reduction of Fe(III) and dissolution of Fe(II) plays a major role in producing Fe isotope variations on Earth and outperforms abiotic redox processes by several orders of magnitude in this regard /JOH 08/. Thus, the microbial reductive dissolution exerts a measurable impact on the geochemistry of natural waters. The major problem with a quantification of the possible impact of this process on clay properties in a DGR would be with the assumption on the maximum size of the population of Fe(III)-reducing microbes at the conditions specific to a DGR after its closure, as no corresponding reliable data appear to exist for indigenous or disturbed clays. Even if the population size may be rather low in the indigenous clay, it is still not known how it can increase upon a disturbance, which is a question of vital importance for assessing long-term performance of a DGR. A clear research demand can therefore be stated for this issue.

Besides the three above conditions necessary for the occurrence of the reductive dissolution, the non-reductive dissolution of clay requires additionally the presence of low-molecular-weight compounds with high affinity to Fe(III). During the building and operation phases of a DGR, siderophores necessary for microbial respiration of Fe(III) can be produced by aerobic and facultative anaerobic microbes populating the layers of claystone adjacent to repository galleries as well as the interfaces in and on the emplaced clay buffer. After the consumption of oxygen in the containment-providing rock zone, only a limited production of siderophores, if any, can be assumed due to conditions less conducive for Fe(III) respiration by facultative anaerobes, as strict anaerobic microbes – becoming then increasingly active – are not known to be able to produce siderophores. However, this further production of siderophores may be unnecessary, as it has been argued that siderophores produced by one microbial species can be utilized for Fe respiration by another one /KAL 00/.

In a Late Eocene (~35 million years old) clay with an organic carbon content of 0.91 ± 0.09 weight %, which is typical for deep clay formations, high contents of acetate (17 ± 3 µmol g^{-1}), formate (4 ± 1 µmol g^{-1}), and pyruvate (0.6 ± 0.3 µmol g^{-1}) have been determined /SHE 05/. Even if concentrations of these chelators and of siderophores in the specific host formation or the buffer material of a DGR might be several orders of magnitude lower, their synergistic effect should still be taken into account, as comparable rates of goethite dissolution have been observed in the presence either of 0.5 µmol ml^{-1} oxalate or >0.1 µmol ml^{-1} siderophore alone or of only 0.04 µmol ml^{-1} oxalate combined with 0.01 µmol ml^{-1} siderophore /CHE 03/. Similarly, an addition of 1 µmol ml^{-1} nitrilotriacetic acid, another weak chelator, has been found to result in a fourfold increase of Fe(III)-reducing activity of microbes in clays /SHE 05/. Furthermore, siderophores function from pH 2 to 12 (in contrast, oxalate does not chelate effectively at neutral to alkaline conditions) /KAL 00/ and are protected from thermal degradation at temperatures of up to 105 °C through adsorption on a smectite /SIE 06/, so that their chelating capability may not be significantly deteriorated at the repository conditions.

Unfortunately, neither the possible production of siderophores during the oxygen availability in a DGR nor the synergistic effect of organic acids and siderophores, if any, nor the possible synergistic effect of organic compounds available in the clay formation and those secreted by microbes inoculated into a DGR has been studied for clays so far. The importance of this issue is highlighted by the observation that kaolinisation of muds deposited during the Late Cretaceous and Early Tertiary period (~40 - 80 million years

ago), which occurs along with microbial dissolution of smectite and illite as sources of Fe(III), is negligible where muds were exposed to (nearly) anoxic water but could be accomplished within a few thousand years upon their exposition to oxic groundwater containing organic chelators /HUR 97/. The latter study has concluded that the world's largest deposit of commercial quality high-brightness kaolin would not have formed without the microbial activity, which resulted in dissolution and reduction of Fe(III) and destruction of organic matter. This activity requires that kaolin products to be delivered in a slurry form be routinely treated with a biocide to reduce microbe reproduction below the industry tolerance level.

Another very important, yet unresolved issue is that of flavins, which are secreted equally efficiently under both aerobic and anaerobic conditions and were discussed in detail for the safety-relevant process "Microbial reduction of clay minerals". Flavin mononucleotide and riboflavin, which are effective electron shuttles, have been shown to strongly adsorb on a smectite (up to ~0.5 mmol g^{-1} of clay) as well as to chelate Fe(III) adsorbed on its surfaces /MOR 83/, /MOR 84/. Furthermore, at least two examples are known of microbes secreting riboflavins to dissolve Fe(III) required in their metabolism /MAR 08/. However, studies of clay dissolution due to flavins, let alone of possible synergistic dissolution effect in combination with organic acids or siderophores, are lacking. It should be noted in this relation that microbial production of chelating compounds in the repository environment was recently judged particularly important in the safety analysis of radioactive waste disposal while being one of the least understood processes /PED 05/. This knowledge state should be obviously regarded as rather unsatisfactory and ought to be improved in future research activity related to the final disposal of radioactive waste.

3.4 Summary of the specifications of the process "Microbial dissolution of clay minerals"

Safety-relevant properties of clay influenced:

(i) swelling pressure (negatively influenced),
(ii) specific surface area (negatively influenced),
(iii) cation exchange capacity (negatively influenced),
(iv) anion sorption capacity (negatively influenced),
(v) porosity (negatively influenced),

(vi) permeability (negatively influenced),
(vii) fluid pressure (negatively influenced),
(viii) plasticity (negatively influenced).

Barriers with safety-relevant containment function affected:

(i) clay buffer,
(ii) claystone.

Mechanisms:

(i) destabilisation of the clay mineral structure upon an extensive reduction of structural Fe(III),
(ii) chelation of structural Fe(III) by dissolved chelating ligands followed by solubilisation of Fe(III).

Reactions involved:

See the selection of reactions given in the summary of the process “Microbial reduction of clay minerals”.

4 Biofilm formation

A water-filled basalt fracture with a width of ~100 µm, located at a depth of 2.8 km in a gold mine in South Africa (water temperature of 52 °C, pH of 9.16, and Eh of −263 mV), has been found to be populated by ~5 × 10^4 microbes per cm^2 of the fracture surface encrusted by a millimetre thick layer of the clay mineral chamosite /WAN 06/. These microbes were attached at the clay mineral–water interface as individual microbes or aggregates of up to five microbes, and the extensive regions of the surface were covered with organic material resembling extracellular polysaccharides (Fig. 4.1 A), which was either embedding or devoid of microbes. These surface-attached, sessile microbes outnumbered by two orders of magnitude the planktonic ones, living in the pore water, and this ratio between sessile and planktonic microbes, observed in the deep terrestrial subsurface is also characteristic of other natural environments /ARN 10/.

Microbes produce various extracellular polymers (primarily polysaccharides but also proteins and nucleic acids) as attachment structures to anchor cells to the surface /BAR 98/. The initial attachment is considered to be readily reversible but to become, over time, strong and irreversible /BEV 97/, as has been conclusively demonstrated for a natural geological setting (Fig. 4.1 B) /WAN 06/.

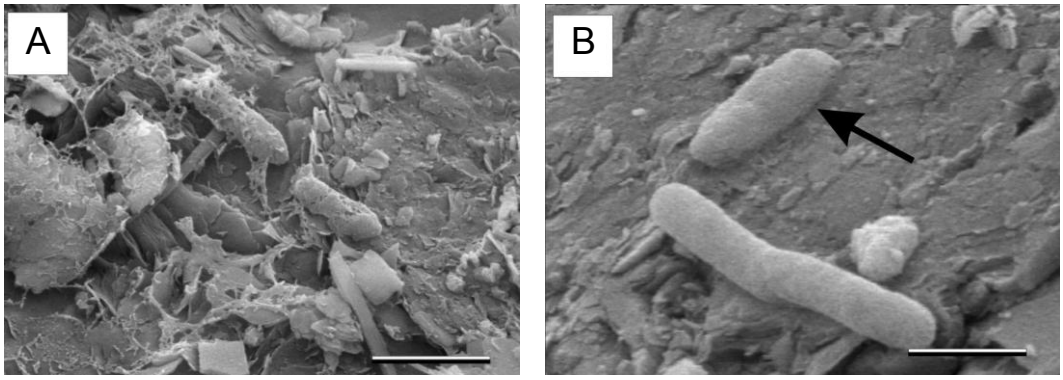

Fig. 4.1 Scanning electron microscopy micrographs of microbes coated with presumably extracellular polysaccharides (A). Some microbes were observed not to be accompanied by such organic coatings (B). Microbes can be attached firmly (arrowed) or loosely to the clay mineral surface (B). Scale bars correspond to 2 µm in (A) and 1 µm in (B) /WAN 06/

It has been observed in a field study carried out in an Antarctic Dry Valley stream that the atomically flat, basal surface of muscovite showed numerous clusters of ~1 nanometre-deep, flat-bottomed etch pits as a result of such strong attachment /MAU 02/. Similar observation of the numerous etch pits and cracks has been made for the surface of feldspar /BAR 98/.

Provided that nutrients are available, this initial colonization of the surface can proceed through division of attached microbes, attachment of further planktonic ones, and continuing secretion of extracellular polymers to eventually develop a biofilm, which can be one up to thousands of micrometres thick and completely cover the original solid surface /BRO 94/, /BEV 97/, /BAR 98/, /AND 06/. Extracellular polymers represent a matrix embedding individual microbes within a biofilm and protecting them from desiccation and other physical, chemical, and biological stresses. A further protection is provided through a thin boundary structure – presumably a lipid bilayer similar to that constituting the microbial cell membrane – enveloping the entire biofilm (Fig. 4.2 A) /PAL 09/.

These protective components give a strong advantage to microbes organised in a biofilm over those in planktonic form. For example, the resilience to flowing supercritical CO_2, which can quickly diffuse into complex cellular material and effectively destroy it, of a six-day-old biofilm is a factor of 100 higher than that of suspended, planktonic microbes /MIT 08/. Similarly, microbes organized in a one-day-old biofilm are by a factor of 600 more resilient to the oxidizing agent hypochlorite (ClO^-) than the same microbes in planktonic form /LUP 02/. In the case of highly penetrating gamma radiation, however, no advantage of three-day-old biofilms over planktonic microbes has been observed for radiation doses of up to 1500 Gy /NIE 07/.

Nevertheless, individual microbes do form biofilms surviving in highly irradiated environments, such as on spent nuclear fuel cladding with a dose rate of 2.1 Gy h^{-1} and the total dose on biofilm microbes of up to 3200 Gy /BRU 09/. In a further striking example, microbial biofilms developed on the walls of the damaged Three Mile Island reactor during its defueling operation in 1986 despite a radiation dose rate of 100 Gy h^{-1} and borate concentrations above 70 mmol l^{-1} in the cooling water /WOL 97/. As a result of the concomitant strong increase of the planktonic microbial population, the visibility of reactor core debris deteriorated below ~20 cm, and the defueling operations had to be ceased.

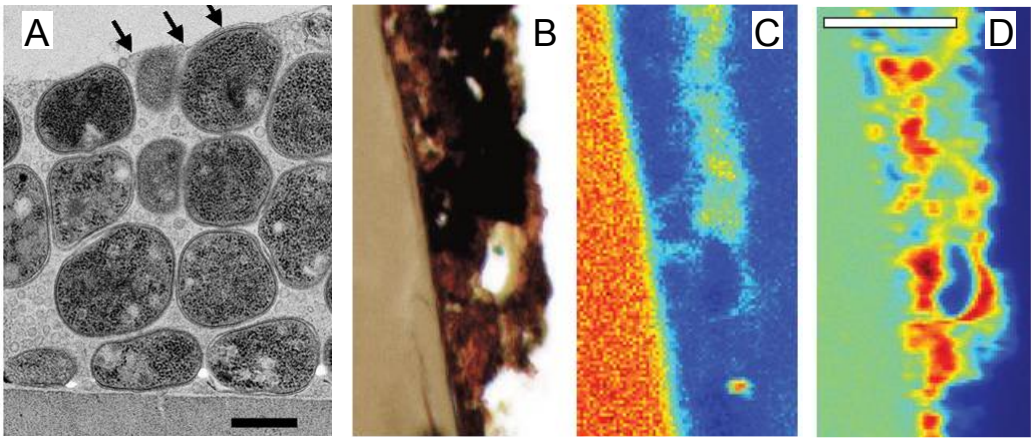

Fig. 4.2 (A) Cross-section of a thin, tightly packed biofilm on a solid substrate. The biofilm boundary with a thickness of ~5 nm at the interface with air is denoted by multiple arrows. The spaces between the microbes, noteworthy adjusting their shapes to accommodate within the biofilm, are occupied by vesicles that are 30 to 60 nm in diameter (scale bar corresponds to 500 nm) /PAL 09/. (B–D) Cross-section of a biofilm grown on the seafloor on a volcanic glass (B) with maps of Ca (C) and Fe (D) distributions (scale bar corresponds to 30 µm) /TEM 09/. In (C), Ca concentrations vary from high (red) in volcanic glass to low (blue) in the contacting solution. Colour scheme for Fe in (D) differentiates Fe(II) (green) versus Fe(III) (red)

Besides the protective functions, the extracellular polymer matrix and the biofilm boundary allow microbes to control the level of metal ions, to which they are exposed due to the contact with the overlying aqueous solution and the underlying mineral surface /BRO 94/, /BAR 98/, /TEM 09b/. Such a control is necessary, since although some metals are essential for growth, others can be toxic. Accordingly, biofilms often contain K^+, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+} at concentrations 2 to 4 orders of magnitude greater than those measured in the contacting aqueous solution with, e.g., Pb^{2+} accumulated in a highly insoluble form as a detoxification measure /TEM 09a/. A natural anaerobic biofilm collected from a borehole ~1.5 km below land surface has been reported to maintain even ~2×10^7 times higher Zn^{2+} (in form of zinc sulphide ZnS) concentrations than the contacting borehole water /MAC 07/. From a lacking correlation between the metals concentrated in the biofilms and the composition of either the solution or the mineral, the accumulation of metals in biofilms has been concluded to be selective and unique for each specific subsurface environment /BRO 94/, /LAP 97/.

Another important advantage of microbial organisation into biofilms is the creation of controllable microenvironments around individual microbial cells, which results in establishing pH, redox, and nutrient gradients within the biofilms /HUN 08/, /TEM 09b/. The latter study has presented a conclusive demonstration of variability of metal concentrations and iron oxidation state, which is controlled by pH and redox potential, within a biofilm on the micrometre scale of individual microbes (Fig. 4.2 B–D). The former study has revealed that multiple iron oxidation states within a biofilm can be generated by individual microbes through conditioning their localized microenvironments to allow extracellular Fe(III) reduction, which occurs then via an electron transfer mechanism (see discussion for the safety-relevant process “Microbial reduction of clay minerals”). Metabolic expense of secreting electron shuttles or Fe(III)-chelators has been demonstrated to strongly decrease in a biofilm, where they can be effectively reused and where their accumulation has been found to lead to a considerable (by ~70 %) increase of the rate of electron transfer to Fe(III) /MAR 08/.

Furthermore, a rapid vertical stratification of Se redox-species across a biofilm of microbes using Se(VI) as a terminal electron acceptor has been observed as a result of microbial reduction of Se(VI) at the aqueous solution–biofilm interface with Se(0) accumulating within the biofilm and Se(IV) diffusing through the biofilm to bind to the underlying mineral surface /TEM 09b/. Despite such a strong influence exerted by biofilms on the solution chemistry at the mineral interface, the observation of Se(IV) adsorption at the mineral–biofilm interface and further experimental evidence discussed in the latter study suggest that the formation of a biofilm may not necessarily block the highly reactive sites on the mineral surfaces. The net effect of biofilm formation on cation and anion sorption capacities of solid substrate (**safety-relevant properties**) depends, however, on the solute identity. Indeed, the adsorption of Co(II), Th(IV) and Np(V) on the granite rock surface has been observed to decrease and that of Pm(III) and Am(III) not to change significantly or even to increase upon the formation of a biofilm under in situ subsurface conditions at the Äspö Hard Rock Laboratory /AND 07/.

The spatial variability of solution chemistry and accumulation of metals within a biofilm often lead to a precipitation of very different mineral phases mediated by subpopulations of microbes. A study of an up to 10 mm thick biofilm formed within 4 to 6 weeks at a depth of 420 m upon excavation of an underground research laboratory in a granitic formation of the Canadian Shield has provided evidence that the biofilm consisted of an oxic zone at the air interface and an anoxic zone at the mineral interface /BRO 94/. As

a result of microbial reduction of Fe(III) in the clay mineral biotite of the granitic host rock and subsequent dissolution of Fe(II), anaerobes mediated precipitation of siderite ($FeCO_3$), whereas Fe(II) migrating to the outer face of the biofilm was oxidized to Fe(III) by aerobes and formed ferrihydrite ($Fe_2O_3 \cdot n\ H_2O$) or hematite (α-Fe_2O_3) precipitates. This mechanism allowed narrow bands of hematite and siderite, characterized by very different stability fields, to exist only millimetres apart. Similarly, a microbially mediated formation of U(V) and U(VI) precipitates in a detoxification process within a biofilm formed on biotite has been observed and attributed to accumulation of U(VI) from the overlying aqueous solution within a subpopulation of microbes concurrent with its reduction to U(V) and accumulation of U(V) within another subpopulation /ARN 10/. The presence of U(V) has been considered in the latter study as an evidence that U(VI) reduction involves a one-electron – and not a two-electron – transfer.

The above discussion on biofilms exemplifies the ability of microbes to create local environments, which are characterized by chemical conditions more conducive to their growth and survival than those in the surrounding bulk environment, through organisation into biofilms. Additionally, microbes can influence chemical conditions of at least parts of the bulk solution adjacent to biofilms. Indeed, biofilms formed on exfoliation planes of biotite grains within two weeks have been observed to plug the few-micrometre-thick pore space between these planes and to increase the acidity in the confined pore solution by 3 to 4 orders of magnitude over the external bulk solution presumably due to production of low-molecular-weight organic acids /BAR 98/. In contrast, no pH gradient was detected in biofilm absence or for relatively small and thin biofilms on the outer surfaces of biotite grains, with the latter observation being attributed to a dilution effect of bulk solution on produced organic acids.

For biotite dissolution, such an observed decrease in solution pH from the bulk value of 7 to the value of 3 inside the biofilm and the confined pore space corresponds to an about twenty-fold increase in dissolution rate /BAR 98/. Conservatively assuming that 10 % of biotite surface were covered by microbial biofilms characterized by the measured pH value of 3, it has been concluded in the latter study that approximately 50 % up to 100 % of the mineral dissolution should be attributed to microbial acid production. Furthermore, differently from predominantly incongruent dissolution of biotite in abiotic experiments at neutral pH, microbial acidification through biofilm formation switches the dissolution process to a congruent mode. This was accompanied by a dramatic in-

crease in Fe release to bulk solution (by up to 0.12 µmol ml^{-1} at the experiment settings) and an overall acceleration of degradation of the mineral structure.

The formation of biofilms has been concluded to substantially influence mass transport and hydrodynamics in porous media following the observation that the porosity of sand with grain size of 0.12 up to 0.7 mm decreased by 90 - 96 % after five days of biofilm growth /CUN 91/. Magnetic resonance measurements carried out at a fixed volumetric flow rate on a model porous media constructed of polystyrene beads have revealed increased regions of no flow due to biofilm-plugged pores, which generated high velocity in unplugged pores to maintain the fixed flow rate (Fig. 4.3) /SEY 04/. This figure demonstrates the sensitivity of transport processes to biofilm growth, as the just discernible amounts of biofilm exert a strong impact on the pore velocity distribution by day 2.

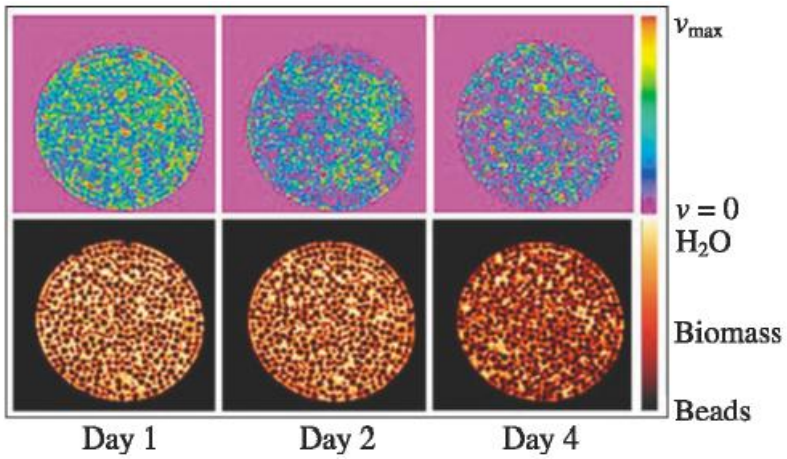

Fig. 4.3 Magnetic resonance maps of velocity (top row) and magnetic relaxation of water molecules (bottom row) as a function of biofilm growth time (left to right) in a column constructed of 0.24 mm polystyrene beads. Spatial resolution is 55 µm per pixel with 128 × 128 pixels in plane. Visualization of biofilm formation and growth is carried out by measuring water relaxation times, which become enhanced (coloured darker in the bottom row) in the biofilm due to the inherently restricted motion of water molecules there /SEY 04/

An additional magnetic resonance method has been applied in the latter study to measure conditional probability of water movement through the polystyrene bead column over time. These measurements have revealed that the biofilm growth alters the hydrodynamics of the system with the probability of small displacements (<250 µm within the measurement time of 0.3 s) strongly increased due to water incorporation into the biofilm and entrapment in plugged pores. A simultaneous moderate increase in the probability of larger displacements (>1750 µm) in high-permeability unplugged pores occurred as required by the imposed fixed flow rate.

Similarly, packed columns of crushed granitic rock from Äspö underground research laboratory flushed by the artificial Äspö groundwater under anaerobic conditions have been reported to become impermeable within two days because of formation of biofilms of Fe(III)-reducing microbes indigenous to the Äspö rocks /TUC 06/. In another case, filter canister designed to remove 0.5 - 800-µm-small fine debris suspended during the defueling operations in the damaged Three Mile Island reactor – in order to maintain water clarity and minimize radiation exposure – have been plugged at abnormally fast rates with very little total solids entrained due to biofilm formation /WOL 97/.

In a study of clay mineral colloid transport through sand columns, an almost complete retention of the colloids with a size of 30 nm has been observed for three-weeks-old biofilms exposed to 20 pore volumes of Ca^{2+} ion influent in advance of a colloid breakthrough measurement using deionized water (Fig. 4.4 A) /MOR 07/. This transport inhibition took place despite a fourfold decrease of microbe number in the column as a result of a partial biofilm detachment during the colloid breakthrough measurement. In contrast, a considerable colloid transport occurred in control, sterile experiments with either Ca^{2+} or Na^{+} ion influents. Moreover, no colloid retention, an enhanced colloid transport, and an eightfold reduction of microbe population in the column have been observed for the colloids as the same biofilms were exposed to 20 pore volumes of Na^{+} ion influent before measuring colloid breakthrough (Fig. 4.4 B).

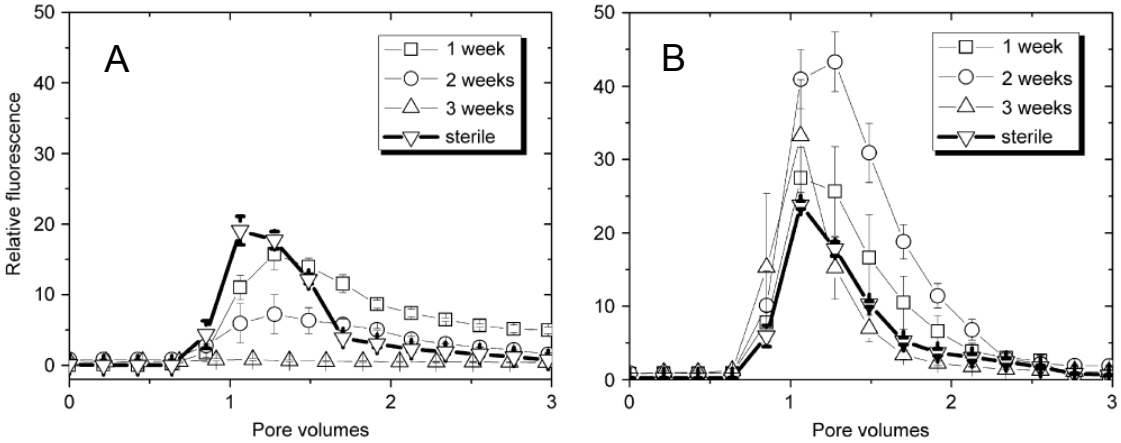

Fig. 4.4 Breakthrough of clay mineral colloids through sand columns in which Ca^{2+} (A) or Na^{+} (B) ions had been predominant before low ionic strength conditions have been established by replacing the salt influent with deionised water after one, two or three weeks of biofilm growth (means and standard deviations calculated from three replicas). Sterile controls have been obtained at the same electrochemical conditions with means and standard deviations calculated from nine replicas /MOR 07/

In conclusion, biofilm formation on a mineral surface, which can occur within a few days, can facilitate clay mineral reduction and dissolution and thus aggravate their negative impact on eight safety-relevant properties discussed for the safety-relevant processes “Microbial reduction of clay minerals” and “Microbial dissolution of clay minerals”. A further major negative effect of biofilm formation concerns metal corrosion and is discussed below for the safety-relevant process “Microbially influenced corrosion”. In an immediate effect, biofilm formation and growth can modify cation exchange or anion sorption capacities (**safety-relevant properties**) of the underlying mineral surface and strongly reduce porosity and permeability (**safety-relevant properties**) of the adjacent pore space.

4.1 Evaluation of the safety relevance of the process “Biofilm formation”

Based on observations of extensive biofilm formations on the walls of access tunnels, ventilation raises or boreholes in the Swedish and Canadian underground research laboratories excavated in granitic formations potentially suitable to host a DGR /BRO 94/, /AND 06/, it can be assumed that similar biofilms can form as a result of deep excavations in a claystone formation. However, although biofilms do form on clay

minerals under laboratory conditions as discussed above, the present study could not find any in situ evidence in support of this assumption in the relevant literature.

Water availability has been identified as the limiting factor for the formation of biofilms of Fe(III)-reducing and sulphate-reducing microbes in granitic environments /BRO 94/, whereas oxygen levels above ~0.1 µg ml^{-1} /AND 06/ and temperatures below 60 °C /ELS 03/ were additionally required for Fe(II)-oxidizing microbes to form biofilms in contact with granite or tuff. The latter two requirements are fulfilled for weathered excavated systems, so that a probable reason for lacking observation of biofilms in deep excavations in claystone formations may be a rock desiccation by the venting as observed for a proposed DGR in a volcanic tuff formation at Yucca Mountain, USA /KIE 97/.

After the repository closure, however, disturbed claystone or clay buffer within the containment-providing rock zone will be inevitably saturated with water from the surrounding, undisturbed claystone and water availability will not limit anaerobic biofilm formation any more. The principal limitation on biofilm formation, growth or survival will be posed by a decrease of available pore space as a result of swelling of compacted clay or claystone occurring upon the water saturation. Nevertheless, the excavation damaged zone, waste canister–clay buffer and clay buffer–host rock interfaces as well as those between blocks or granules of clay buffer can form environments conducive to microbes in a DGR because of the associated decreased material density and increased water availability /DEC 04/, /KUR 04/, /AER 09a/, /AER 09b/, /STR 11/, /WER 11a/.

Indeed, a biofilm formation of anaerobic sulphate-reducing microbes has been documented at the interface between crushed claystone, originating from Callovo-Oxfordian clay formation considered to host a DGR in France, and a carbon steel, potentially be used as canister material /ELH 10/. Although that study has not given a discussion on biofilm formation in the second series of the experiments with undisturbed claystone cores carried out at the in situ pressure of 12 MPa, microbial corrosion effect on the steel was very similar for both, crushed and undisturbed claystones. Similarly, the number of sulphate-reducing microbes on steel tubes deposited for 20 years in Boom Clay, which is considered as the reference host rock in Belgium, has been revealed to be higher by a factor of up to ~10^5 than that at a distance of only 2 cm away from the clay–steel interface /AER 09a/.

No discussion on biofilm formation has been given in the latter study either. However, the ratio of ~10^5 strongly exceeds that of 5 × 10^3 observed between the populations of a two-month-old biofilm and planktonic microbes in the incoming groundwater at Äspö underground research laboratory /AND 06/. Since sulphate-reducing microbes in the clay buffer reside most likely not as planktonic but rather as surface-attached ones, this difference (by a factor of ~10^5 / [5 × 10^3] = ~20) becomes even more noticeable (by a factor of ~2 × 10^3) taking into account that surface-attached microbes outnumber planktonic ones by two orders of magnitude (see discussion at the beginning of the current section). Despite these indications of possible biofilm formation in a clay buffer, no compelling evidence substantiating or refuting such formation has been presented so far.

Furthermore, although the temperature limit of 60 °C has been observed for a biofilm formation by aerobic microbes native to the subsurface tuff at Yucca Mountain /ELS 03/, anaerobic sulphate-reducing microbes retrieved from deeply buried (1 to 4.6 km) oil reservoirs in the North Sea have been reported to form biofilms on mineral surfaces at temperatures of 80 °C /ROS 91a/ and even 90 °C (no visible extracellular polymer material was associated with biofilms in this case though) /SPA 00/. Moreover, anaerobic sulphate-reducing microbes, which do not form biofilms at the conditions of their natural habitat, can form biofilms in response to environmental stress /LAP 97/. Microbes reported on in the latter study thrived as planktonic cells at temperatures between 60 to 86 °C but formed biofilms upon a temperature increase to 100 °C or a decrease below 60 °C.

Therefore, the lacking observation of biofilms in excavations of claystone formations cannot be considered to suffice for the exclusion of possible biofilm effects when carrying out an assessment of the performance of a DGR in the post-closure phase. The DGR closure will lead to changes in the clay buffer and the disturbed claystone environments, which may exert stress on indigenous or inoculated anaerobic microbial communities and foster their organisation into biofilms. Although general effects of biofilm formation on survival and growth of microbial communities as well as on properties of mineral surfaces and pore solutions in the deep subsurface become increasingly known, the specific impact of biofilms on DGR components and host material within the containment-providing rock zone remains to be identified and quantified in future research.

4.2 Summary of the specifications of the process “Biofilm formation”

Safety-relevant properties of clay influenced:

(i) swelling pressure (negatively influenced),
(ii) specific surface area (negatively influenced),
(iii) cation exchange capacity (sign of influence depends on the solute identity),
(iv) anion sorption capacity (sign of influence depends on the solute identity),
(v) porosity (negatively influenced),
(vi) permeability (negatively influenced),
(vii) fluid pressure (negatively influenced),
(viii) plasticity (negatively influenced).

Barriers with safety-relevant containment function affected:

(i) waste canisters,
(ii) clay buffer,
(iii) claystone.

5 Microbially influenced corrosion and activity of sulphate-reducing bacteria

A transfer of electrons from zero-valent metal to an external electron acceptor, which results in release of the metal ions into the contact solution and deterioration of the metal, is termed corrosion /BEE 04/. An acceleration of this electrochemical process due to microbial activity is referred to as microbially influenced corrosion (MIC). The major concern with MIC issues with respect to final disposal of radioactive waste is related to a potential loss of the safety-relevant containment function of steel or copper canisters or over-packs, which are aimed at preventing water contact with waste and release of radionuclides, well in advance of the supposed a few thousand up to hundred thousand years. Indeed, while rates of abiotic, anaerobic iron corrosion by water attack (primary dissolution $Fe \leftrightarrows Fe^{2+} + 2e^-$ followed by water dissociation and reduction of derived protons $2e^- + 2H^+ \rightarrow 2H_{(adsorbed)} \rightarrow H_{2(adsorbed)} \rightarrow H_{2(aqueous)}$) are expected to be <10 µm yr^{-1}, as high anaerobic corrosion rates as of 700 µm yr^{-1} can be observed in the case of MIC /SHE 11/.

Anaerobic MIC is most closely identified with sulphate-reducing bacteria (SRB) and is currently considered to proceed according to the following major mechanisms in dependence on the involved microbial species or environmental conditions: (a) an indirect mechanism by hydrogen sulphide (H_2S) attack, (b) an indirect mechanism by adsorption of microbially produced extracellular polymers at the metal–water interface, (c) a direct mechanism by scavenging of hydrogen atoms accumulating on the metal surface as a result of corrosion, and (d) a direct mechanism by an uptake of metal electrons into microbial cells.

(a) Hydrogen sulphide attack occurs according to reaction $M + H_2S \rightarrow MS + H_2$ for cast iron, stainless or carbon steel (M = Fe) or copper (M = Cu) and in anaerobic environments is attributed to activity of sulphate-reducing bacteria, which release hydrogen sulphide as a metabolic by-product /DIN 04/. This mechanism does not generally require a direct contact between SRB and the metal surface. Indeed, results of a recent study strongly indicate that corrosion of copper plates buried within a clay buffer compacted to as high a density as of 2.0 g cm^{-3} occurred as a result of sulphide production by SRB at the clay buffer–groundwater interface, from where

sulphide diffused to the copper surface with a diffusion coefficient[10] of 0.2×10^{-11} m^2 s^{-1} /PED 10/. Since sulphate diffuses into the clay buffer as well, so that its pore water concentration increases up to 50 - 70 % of that in groundwater /PED 10/, sulphide can be also produced by SRB populating the clay buffer. However, the rate of such sulphide production strongly depends on the clay density and has been found to decrease by a factor of ~50 upon clay density increase from 1.5 g cm^{-3} to 2.0 g cm^{-3} /MAS 10a/.

In a further negative effect, hydrogen sulphide accelerates the rate of metal cracking by kinetically inhibiting combination of the hydrogen atoms produced at the metal surface to H_2 and permitting a large fraction of the hydrogen atoms to enter the metal /BER 82/. The latter study refers to the steels heat-treated to strengths above ~6×10^8 N m^{-2} – as required for drill pipe and production tubular in oil and gas industries – as most affected with the failures occurring well below the yield strength of steel.

Yet, practically all metal materials, high tensile or ductile amongst others, can be damaged as a result of the absorption of a sufficient quantity of hydrogen /WOO 00/. The hydrogen dissolved in steel is characterized by a diffusion coefficient of 10^{-8} - 10^{-9} m^2 s^{-1}, which is comparable to that in aqueous solution, and can penetrate steel to a depth of up to 100 µm /WOO 00/. The basic hydrogen-steel interaction is an easing of dislocation generation and motion, so that the structure deformation processes are greatly aided by the presence of hydrogen dissolved in the lattice ahead of the crack tip /BEA 72/. Furthermore, hydrogen penetration can cause losses of steel strength by structural changes, such as decarburization in carbon steels due to reaction of steel carbon with the hydrogen to form methane /WOO 00/.

(b) Formation of a film of porous ferrous sulphide deposits on a corroding steel surface following the hydrogen sulphide attack can partially passivate the surface, so that the corrosion rate remains as low as that for the water attack (<10 µm yr^{-1}) despite increasing concentrations of sulphide /SHE 11/. However, this passivation takes place only for abiotic sources of sulphide (e.g., Na_2S) and not in the presence of SRB actively producing sulphide /BEE 99/, /SHE 11/. This microbial effect has been

[10] Unfortunately, there is no clear statement on the kind of the determined diffusion coefficient (in pure water, effective or apparent) in the original paper.

concluded to be most likely due to extracellular polymers embedded into the ferrous sulphide film (Fig. 5.1 A) and facilitating electron transfer from the metal to a terminal electron acceptor /BEE 04/. An adsorption of as little as 10^{-8} g cm^{-2} of extracellular polymers produced by microbes in order to attach to a surface (see discussion for the safety-relevant process “Biofilm formation”) can deteriorate corrosion protective properties of a passive film on stainless steel /BEE 99/.

As soon as extracellular polymers are secreted by microbes into the solution contacting the metal surface, the further presence of microbes may become unessential for corrosion to occur /STA 08/. As low concentrations of extracellular polymers as of <30 mg l^{-1}, which were harvested from SRB in a separate medium, have been found in the latter study to be able to induce a corrosion of cast iron and carbon steel within one day. Nevertheless, extracellular polymers secreted by one of the three studied SRB species have not induced corrosion at all. Moreover, adsorption of extracellular polymers secreted by a thermophilic SRB species, preferentially growing at 50 °C, has been observed to even slow down the corrosion process and lead to a twofold reduction of electron flow from corroding steel into contacting solution /ANA 09/. The reasons behind these different effects of extracellular polymers on metal corrosion remain to be established.

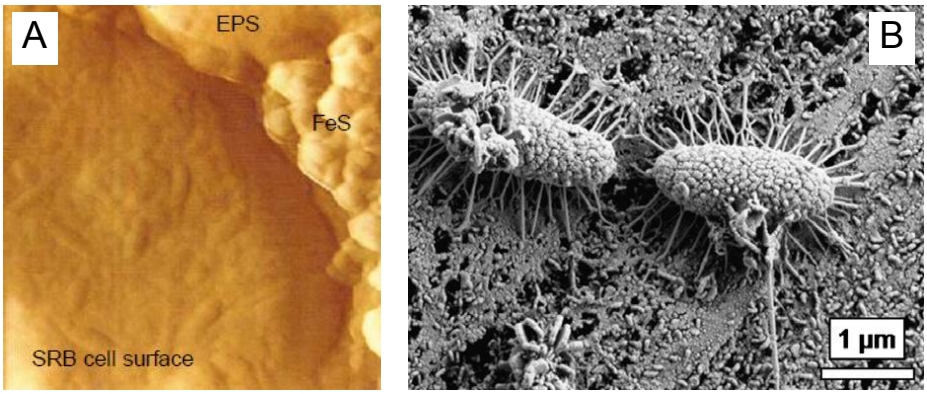

Fig. 5.1 Atomic force microscopy micrograph of ferrous sulphide (FeS) particles associated with a sulphate-reducing bacterium (SRB) and extracellular polymers (EPS) on the surface of a stainless steel (micrograph width corresponds to 1.14 µm) (A) /BEE 04/. Scanning electron microscopy micrograph of individual SRB heavily incrusted with ferrous sulphide particles and firmly attached to the surface of a carbon steel by an extensive array of organic filaments (B) /SHE 11/

(c) During a water attack, electrons released from metal reduce dissociated protons to produce hydrogen atoms adsorbed on metal as shown above. Their combination to H_2, which can subsequently desorb from the metal surface, is considered as the step that limits the rate of the overall corrosion reaction in anoxic sterile water /DIN 04/. This step – and accordingly the entire corrosion reaction – is accelerated by metal-attached SRB, which utilize adsorbed hydrogen atoms as electron donors to reduce dissolved sulphate according to reaction $8H_{(adsorbed)} + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$ and make reactive sites on the metal surface again available for water attack /DIN 04/.

A hydrogenase enzyme has been shown to be used by SRB to transport hydrogen atoms from the metal surface to the sulphate reduction enzymes /BEE 04/. Furthermore, specific wired structures grown by SRB on steel in the absence of a carbon-based electron source (Fig. 5.1 B) have been proposed as a possible means to directly capture either adsorbed hydrogen (the current mechanism) or electrons from the steel (the second direct mechanism considered below) /SHE 11/. Importantly, hydrogenase can effectively transfer electrons from the steel surface even in the absence of viable microbes /BEE 04/.

(d) Some SRB species have been recently revealed to reduce sulphate in the presence of metallic iron much faster than hydrogen-scavenging SRB species due to presumably a direct electron transport between the metal and microbe via an yet unidentified cell-surface-associated redox-active component /DIN 04/. Such a system, if proved to exist, represents a counterpart to the systems developed by iron-reducing microbes to transport electrons to solid-phase Fe(III) as discussed for the safety-relevant process “Microbial reduction of clay minerals”. A candidate structure has been suggested for this role (Fig. 5.1 B) /SHE 11/ and indeed strongly resembles the electron conducting nanowires grown by iron-reducing microbes (see Fig. 2.1 B). It has been highlighted that the reason why SRB microbes possess a system utilizing metallic iron as electron donor is unknown, since metallic iron naturally occurs only in meteorites /DIN 04/.

Since either of the above corrosion mechanisms requires sulphate to be available as a terminal electron acceptor to SRB and is caused or accompanied by the production of sulphide, a presence of newly-formed sulphide minerals on the corroding metal surface or dissolved hydrogen sulphide in the contacting solution at the anaerobic conditions is

considered characteristic of SRB activity and, as such, of MIC. Based on these indicators, SRB activity has been concluded to have doubled the corrosion rate of carbon steel to ~30 µm yr^{-1} in 20 % suspensions of Callovo-Oxfordian Clay in synthetic pore water as compared to sterile experiments /ELH 10/. This activity led to the formation of a SRB biofilm and of corrosion pits in the carbon steel within one month (Fig. 5.2 A). Furthermore, with undisturbed cores of Callovo-Oxfordian claystone and at the in situ pressure of 12 MPa, an increase of the corrosion rate from a value of 11 µm yr^{-1} in the sterile control to 27 µm yr^{-1} in the presence of SRB has been observed as accompanied by consumption of sulphate – supplied with the inlet synthetic pore water – and production of sulphide.

A recent study with commercial Wyoming MX-80 bentonite clay has revealed that the clay itself is a source of SRB and that a treatment of the bentonite at 120 °C for 15 hours did not sterilize the bentonite though did reduce the number of viable SRB /MAS 10a/. The reduction of SRB population resulted in a decrease of copper corrosion rate by 4 or 16 times for clay densities of 1.8 g cm^{-3} or 2.0 g cm^{-3}, respectively. The survival of SRB in the air-dry bentonite with an initial water content of ~10 % at 20 °C, which corresponds to a water activity of 0.55 - 0.75, and at an even much lower water content during the heat treatment at 120 °C has been attributed by the authors to the formation of spores by SRB. Indeed, a five-year in situ experiment has provided evidences for SRB activity and the presence of spore-forming groundwater SRB in the clay compacted to a density of 2.0 g cm^{-3} and infiltrated by groundwater at a depth of ~450 m in the Äspö hard rock laboratory /CHI 08/.

A further support for the ability of SRB to survive at as high temperature as of 120 °C comes from the observation that spores of thermophilic SRB (Fig. 5.2 B), isolated from oil field waters originating from porous rock formations 2 to 4 km below the seafloor in the North Sea, were highly heat resistant and viable after exposure to 131 °C for 20 min /ROS 91b/. Moreover, SRB have been found to actively reduce sulphate in the hydrothermal deep-sea sediments with optima at 80 to 90 °C and 103 to 106 °C /JØR 92/. Although further detailed studies have neither confirmed nor rejected the ability of SRB to reduce sulphate at temperatures above 100 °C, the optimum temperature range of 80 - 95 °C for SRB activity has been successfully reproduced /WEB 02/.

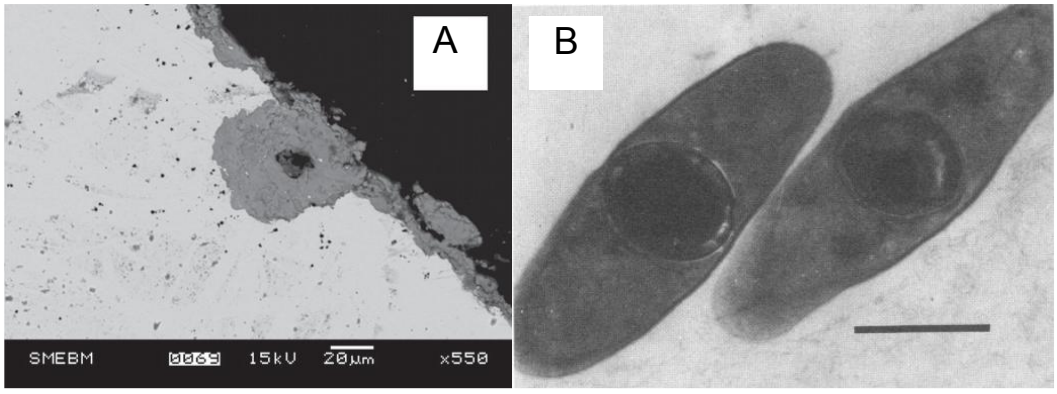

Fig. 5.2 Scanning electron microscopy micrograph of a cross-section of the carbon steel surface with a corrosion pit filled with ferrous sulphide deposit as a result of SRB activity (scale bar corresponds to 20 µm) (A) /ELH 10/. Scanning electron microscopy micrograph of thermophilic SRB cells with spherical spores formed at elevated temperatures (scale bar corresponds to 1 µm) (B) /ROS 91b/

A compelling evidence of the SRB activity in a disturbed deep clay formation has been obtained very recently in an in situ experiment carried out in the Opalinus Clay in the Mont Terri underground research laboratory. In this experiment, synthetic pore water had been circulating in a borehole for five years /WER 11a/. As a result of an unintentional placement of a degradable source of organic carbon (presumably, the highly soluble glycerol[11] from gel-filling of reference electrodes used for a continuous monitoring of redox potential and pH of the borehole solution), a substantial SRB activity has developed in the borehole solution and adjacent clay within a few months after the start of the experiment. This activity led to a more than threefold decrease of sulphate concentration from the synthetic pore water value of 14.7 mmol l^{-1} to 4.3 mmol l^{-1} at the end of the experiment – despite a continuous in-diffusion of sulphate from the surrounding claystone – and an intermediate increase of sulphide concentration to 1.0 mmol l^{-1}, which did not exceed this value due to observed precipitation of ferrous sulphides /STR 11/, /WER 11a/. This experiment has revealed the presence in clay of a spore-forming SRB species and, quite unexpectedly for a rather cold (~30 °C) clay environment, of hyperthermophilic SRB, which thrived in the clay samples exposed to a temperature of 80 °C /STR 11/.

[11] The graphite tip of an electrode has been almost completely leached in this experiment /WER 11a/, which may indicate microbial utilization of graphite /GRE 04/ as well.

Another conclusive demonstration of a potential SRB impact on performance of metals at repository conditions has been provided by a five-year-long mock-up experiment simulating the waste disposal architecture pursued in Belgium. In this experiment, a heat-generating 0.55-m-diameter stainless[12] steel tube has been surrounded by a 72.5-cm-thick layer of pre-compacted blocks of clay-based backfill material[13] and placed within a stainless steel lining /VER 03/. To simulate backfill hydration, which will inevitably occur in a clay host rock, hydration tubes have been added at the interface between the backfill and the steel lining and supplied water from an external water injection system. The dry density of the backfill varied from ~2.0 g cm^{-3} at the surface of the internal tube to ~1.8 g cm^{-3} at the surface of the steel lining, and its water saturation reached the values of 90 - 96 % and 96 - 98 % at these respective locations at the end of the experiment, whereas the maximum temperatures were 137 °C and 117 °C there. Temperatures within the whole backfill were below, e.g., 80 °C only during the initial, six-months-long hydration phase and the last one month before dismantling, and they were above 100 °C for about four years.

Despite the high density and temperature as well as the incomplete water saturation of the backfill, strong indications of MIC have been observed on multiple locations of the lining and, most notably, of the heat-generating tube most distant from hydration tubes /KUR 04/. In one location at the interface between the lining and the backfill, a ~450-µm-thick deposition layer composed of presumably a mixture of chromium oxides (Cr_2O_3) and chromium sulphides (Cr_2S_3) formed with severe, up to 150-µm-deep pits underneath (Fig. 5.3 A–C). A similar formation of chromium sulphide on stainless steel upon exposure to SRB has been observed in other studies and suggested to occur as a result of a reaction of chromium oxide layer – passivating the steel surface against corrosion – with SRB-produced H_2S /CHE 97/, /DUA 06/. In another location at the interface between the tube and sand[14], thick granular deposition formed from nanoparticles consisting of iron and sulphur (Fig. 5.3 D–F). Since only metallic iron and ferrous ions were found to be present in the deposition /KUR 04/, these nanoparticles were

[12] The OPHELIE experiment had been put into operation in 1997; in the current reference repository design, carbon steel is used instead of the stainless steel /OND 04/.

[13] The backfill represented a mixture of sedimentary FoCa clay from the Paris Basin (60 %), pure quartz sand (35 %), and graphite (5 %) /KUR 04/, /VAN 09/.

[14] In the part of the mock-up closest to its main cover, the middle ring of clay-based backfill blocks was replaced by the concrete segmented ring and the inner ring was replaced by sand, whereas the outer ring consisted of backfill blocks with an increased clay content of 85 % /VER 03/, /VAN 09/.

most likely of ferrous sulphide, which is characteristic of MIC induced by SRB as discussed above.

Although up to 5×10^3 thermophilic microbes per g backfill could be cultured at 80 °C after the experiment, SRB presence there has not been reported /DEC 04/. In contrast, more than 10^3 SRB per ml solution and the precipitation of zinc sulphides has been determined in the hydration circuit maintained in the direct contact with pore water of the backfill. As high sulphide concentration as of ~0.6 mmol l^{-1} has been measured in the hydration circuit water, which is comparable to the value measured in borehole solution of the in situ experiment in the Opalinus Clay as discussed above.

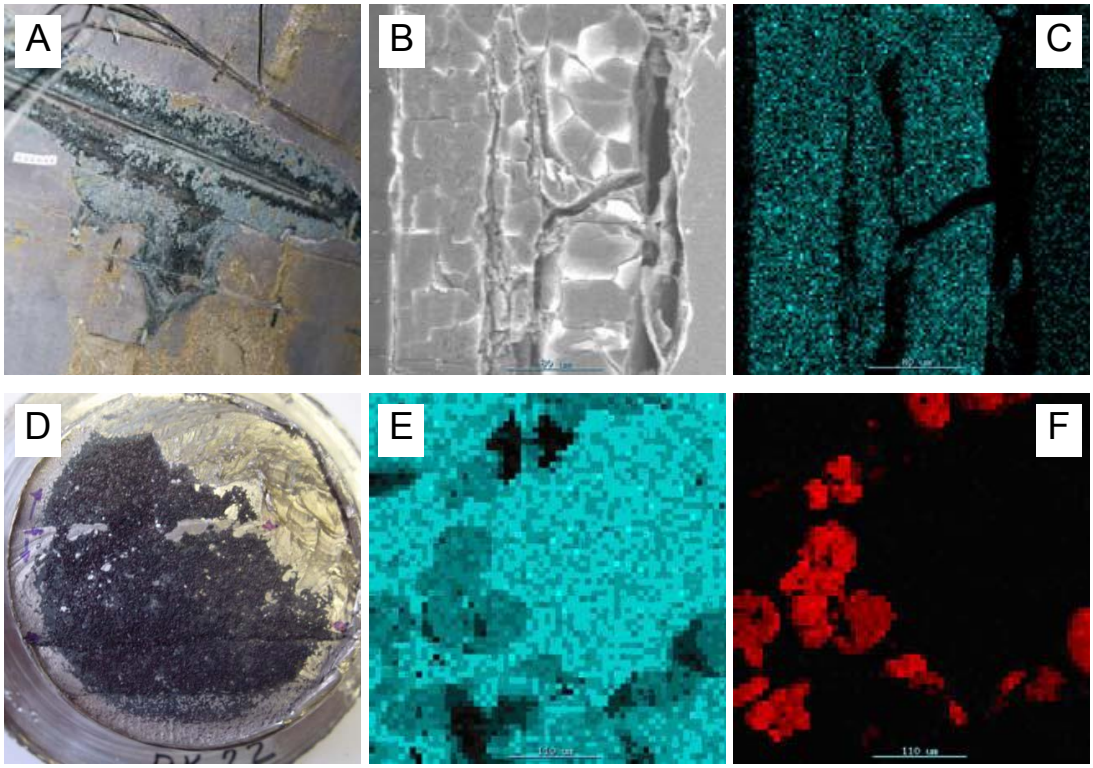

Fig. 5.3 Locations indicative of MIC on the lining (A) and the heat-generating tube (D) after the five-year-long contact with backfill consisting to 60 % of clay and with sand, respectively. Scanning electron microscopy micrograph (B) and energy-dispersive X-ray spectroscopy map of sulphur (C) across the interface between a stainless steel and the deposit from (A). Energy-dispersive X-ray spectroscopy maps of iron (E) and sulphur (F) in the deposit from (D) /KUR 04/

The presence of viable SRB in Boom Clay has been proved from the detection of SRB-specific genes in pore water sampled below HADES underground research facility located at a depth of 224 m below the surface, and the activity of SRB was evidenced by sulphide concentration in the pore water of 4.7 µmol l^{-1}, which strongly exceeds that of 0.01 - 0.1 µmol l^{-1} expected due to the equilibrium with pyrite /DEC 04/, /AER 09b/. Moreover, extensive amounts of SRB cells microscopically have been observed, and as high sulphide concentration as of 5.6 mmol l^{-1} – obviously due to the activity of SRB – has been measured in the pore water extracted from the interstitial space between lining cement blocks of the HADES tunnel and surrounding Boom Clay /LYD 10/.

The latter work reports sulphate concentrations of up to 33 mmol l^{-1} in the pore water which more than sufficed for maintaining such a high SRB activity. Importantly, up to 75 % of this activity has been concluded to result from the use of H_2 as electron donor with the remainder contributed by the abundant lactate (compare reactions (5.1) and (5.3) given in the summary of the specifications of the safety-relevant process “Microbially influenced corrosion and activity of sulphate-reducing bacteria” below). The hydrogen ion consumption associated with the use of H_2 as electron donor by SRB led to an increase of pH value by up to 0.8 units at the applied solid-liquid ratio of 500 g l^{-1} /LYD 10/.

The SRB activity can, however, be inhomogeneous throughout the Boom Clay formation, as no sulphide could be detected in samples taken at distances of 0.4 m up to 7.3 m from a gallery excavated in the HADES facility despite availability of sulphate at concentrations of up to ~4 mmol l^{-1} /AER 09b/. Yet on another location in the HADES facility, up to 4×10^6 SRB per g clay have been found at the interface with steel tubes exposed to Boom Clay for over 20 years, but the number of SRB decreased rapidly with the distance from the interface with less than 40 SRB detected per g clay ~2 m farther from tubes /AER 09a/. Despite severe corrosion of the studied steel samples, only trace amounts of sulphide have been determined on the steel surface, which excludes MIC as a result of SRB activity in this case.

It is important to note that SRB do not depend on sulphate for growth and are able to grow fermentatively on organic acids /ROS 91b/. Some SRB species can grow by reducing nitrate and nitrite to ammonium or by splitting of thiosulphate ($S_2O_3^{2-}$), sulphite (SO_3^{2-}), and sulphur (S^0) into more oxidized sulphate and more reduced sulphide /MUY 08/. Out of the latter five sources of energy for SRB growth, thiosulphate is prob-

ably the most relevant for a DGR for HLW/SF in clays. Thiosulphate concentrations of 0.02 mmol l^{-1} and 0.27 mmol l^{-1} have been measured in the hydration circuit water of the OPHELIE mock-up /VAN 09/ and the in situ experiment carried out in the Opalinus Clay /WER 11a/, respectively. Although no thiosulphate could be detected in clay samples taken at distances of 0.4 m up to 7.3 m from a gallery excavated in the HADES facility /AER 09b/, as high thiosulphate concentrations as of 2.2 mmol l^{-1} have been measured between cement tunnel lining and surrounding Boom Clay /LYD 10/.

Thiosulphate is produced as a main intermediate during the abiotic or microbial oxidation of sulphide minerals (e.g., pyrite), which are often abundant in clays, and may be the major end product of pyrite oxidation if Fe(III) is not available /LUT 87/, /REI 07/. It can also be produced as a result of sulphite reduction by at least one SRB species /REI 07/. A very recent study additionally reports on a thermophilic SRB species reducing sulphate to sulphide at 60 °C but increasingly producing thiosulphate instead of sulphide at lower temperatures /ZHA 12/. Whereas the produced thiosulphate was subsequently reduced by this SRB species to sulphide at 40 and 50 °C, this did not occur at 30 °C. The latter work has further suggested a correlation between the main products of the activity of this SRB species and the changes in the corrosion rates of carbon steel with maximum corrosion observed at 60 °C and the corrosion inhibition observed at 30 °C.

Although the latter results indicate that thiosulphate may not contribute to the corrosion of carbon steel, its role in the corrosion of stainless steel is still a subject of scientific controversy documented, e.g., in /LAY 97/ and /WIL 10/. Some researchers support the view that thiosulphate is a critical species for trigging pit initiation – at the interface between sulphide inclusions and metal in the steel – and stabilising pit propagation, whereas other attribute this critical role to hydrogen sulphide. A common denominator of these different views may be that sulphur adsorbed on an exposed metal surface and resulting from the presence of either sulphide or thiosulphate is the critical species for pitting corrosion of metal, as it accelerates anodic dissolution of stainless steel in acid by strongly bonding to the metal atoms in the lattice and weakening the metal–metal bonds /WER 98/, /WIL 10/.

Thereby, sulphide oxidation to sulphur in the pit, rather than to thiosulphate in the bulk electrolyte (with subsequent electromigration to the pit), is regarded as the predominant step under anoxic conditions, whereas under aerated conditions sulphide oxidation to

thiosulphate is considered to make a contribution /WER 98/. Although initiation of pitting corrosion in stainless steel may be actually due to the chemistry of sulphide inclusions in the steel and not due to the solution chemistry in contact with that /WIL 10/, the question on influence of aqueous thiosulphate cannot be conclusively answered at the current state of knowledge. Yet, thiosulphate concentrations below 5 mmol l^{-1} have been shown to have little effect on the pitting potential of the 316L stainless steel /LAY 97/, which is the reference material for buffer liner in the Supercontainer design in the Belgian concept of disposal of HLW/SF /OND 04/ and was the material of hydration tubes in the OPHELIE mock-up /VAN 09/.

Notably, the experimental evidence suggests that essentially the same SRB species, which can use sulphite and thiosulphate as electron acceptors in addition to sulphate, can be present in subsurface clays at the very different geographical locations (Boom Clay in Belgium, Wyoming bentonite in USA, and in both fresh and saltwater in Africa) /LYD 10/, /MAS 10b/. Similar observations have been made for, e.g., fermentative and methanogenic microbes as discussed for the safety-relevant process "Microbial gas production and conversion" below. Importantly too, Fe(III)-reducing microbes – amongst others the species which forms bacterial nanowires to access insoluble Fe(III) as discussed in the section "Microbial reduction of clay minerals" for Fig. 2.1 and has been shown to use clay Fe(III), U(VI), and Tc(VII) as electron acceptors – can reduce thiosulphate to hydrogen sulphide /VEN 99/, /BUR 09/.

Furthermore and even more importantly for a DGR for HLW/SF in clays, SRB are able to use structural Fe(III) in clays as a terminal electron acceptor, which may explain widespread distribution of SRB in the sub-surface environments, or even to anaerobically oxidize Fe(II) when nitrate becomes available as a terminal electron acceptor (in this case, SRB-produced nitrite further chemically oxidizes Fe(II)) /SHE 03/. With clay Fe(III) as the sole terminal acceptor, the population of SRB has been found to reach as a high level as of ~10^{11} cells per g clay after one week of incubation in a ~7 g l^{-1} clay suspension[15], which was accompanied by a release of ~0.4 mmol of Fe(II) per g clay as a result of reduction of structural Fe(III) by SRB /LI 04/. In the presence of 50 mmol l^{-1} sulphate, the number of SRB doubled, whereas the amount of released Fe(II)

[15] /LI 04/ specifies the Fe(III) concentration of ~30 mmol l^{-1} in the applied clay mineral (reference nontronite NAu-2) suspension without further specifying its solid-liquid ratio. To be however able to quantify the reported Fe(II) releases in relation to the clay mineral mass, the solid-liquid ratio was estimated here based on the specified Fe(III) concentration and the content of ~4.2 mmol of Fe(III) per g of the reference nontronite NAu-2 reported elsewhere /JAI 08/.

tripled to ~1.2 mmol per g clay with estimated 50 % of clay particles being destroyed due to interaction with, most likely, hydrogen sulphide and extracellular polymers as by-products of SRB metabolism. In contrast, clay interaction with the inorganic sulphide (Na_2S) led to a release of ~0.3 mmol of Fe(II) per g clay in the latter study. This increased dissolution effect of biogenic sulphide as compared to abiotic sulphide is in line with the observations made for metal corrosion and discussed above for mechanism (b) of MIC. In a further similarity to MIC, the study /LI 04/ has reported a formation of copious amounts of ferrous sulphide nanoparticles on clay surfaces.

In natural environments, biofilms on corroding metal surfaces often consist of multiple microbial species, metabolism of which occurs in a co-operative manner /BEE 99/, /BEE 04/. Such a co-operation can either facilitate or inhibit corrosion. The corrosion inhibition can occur when corrosion-facilitating – e.g., SRB – and corrosion-inhibiting microbes – e.g., Fe(III)-reducing ones – co-exist and thrive in a common biofilm /BRO 94/, /LEE 06b/. In the latter study, Fe(III)-reducing microbes inoculated anaerobically with SRB at a ratio of 10:1 have been found to inhibit corrosion of a carbon steel in the presence of solid-state Fe(III). This inhibition effect has been attributed to the observed higher production of Fe(II) – from the solid-state Fe(III) – by Fe(III)-reducing microbes and the resulting observed higher thickness of a protective film of ferrous sulphide on the steel surface (see the above discussion for the mechanism (b) of MIC) following the sulphide production by SRB.

However, the observed inhibition effect of Fe(III)-reducing microbes may be specific either to their certain species or to certain composition of community biofilms. Indeed, biofilms consisting of fermentative, extracellular polymer-producing, nitrate-reducing, Fe(III)-reducing, and sulphate-reducing microbes have been found to increase corrosion rates of a carbon steel by a factor of up to five as compared to the sterile controls with a particular contribution due to Fe(III)-reducing microbes /VAL 03/. In another study, an inhibition of pitting corrosion as a result of formation of a biofilm from SRB has been observed as compared to abiotic experimental settings at a similar level of hydrogen sulphide /WER 98/. No discussion on a co-existence of other microbial species in this biofilm has been given in the latter case, but the buffering influence of carboxylate functional groups within extracellular polymers constituting the biofilm has been suggested as a possible explanation of the observation. If it has been indeed a pure SRB-biofilm, this experiment can be considered an example for inhibitive action of adsorbed extracellular polymers as discussed above for the mechanism (b) of MIC.

Even if microbial species co-existing with SRB in a biofilm do not participate in corrosion processes directly, they can still facilitate MIC by maintaining conditions conducive to SRB at the metal surface. Although the most SRB are strictly anaerobic microbes /MUY 08/, they are commonly isolated from natural biofilms in oxygenated environments /BEE 04/. As discussed above for safety-relevant process “Biofilm formation”, a biofilm formed at a depth of 420 m upon excavation of the AECL’s underground research laboratory in a granitic formation. This biofilm consisted of an anoxic zone at the biofilm–mineral interface, where Fe(III)-reducing microbes and SRB were simultaneously active, and an oxic zone at the biofilm–air interface, where aerobic microbial species were active /BRO 94/. At the boundary between these two biofilm zones, hydrogen sulphide was re-oxidized to sulphate, which prevented its escape to the overlying water. In this case, the rate of oxygen diffusion into the biofilm was apparently lower than the aerobic respiration rate within the oxic biofilm zone, which allowed the existence of anaerobic conditions in an aerated environment.

If, furthermore, a microbial species constituting a biofilm together with SRB is corrosion-facilitating itself and directly participates in biotransformation of the metal surface, a synergistic corrosion effect can occur. Aerobic iron-oxidizing microbes are known to produce deposits of Fe(III) oxides and hydroxides on stainless and carbon steels by oxidizing Fe(II) ions released as a result of metal dissolution, below which oxygen becomes depleted /BEE 99/, /RAO 00/. This leads to facilitated oxygen reduction by electrons released from metal outside the oxygen-depleted areas and release of Fe(II) ions underneath the deposits, which results in formation of corrosion pits and build-up of positive charge there. The latter study has further suggested that iron-oxidizing microbes thrive at the deposit–solution interface, where oxygen and ferrous ions are readily available, whereas the metal–deposit interface becomes increasingly anaerobic in favour of SRB growth, which further facilitates pitting corrosion. This synergistic corrosion effect has been confirmed in a recent study, which has found that corrosion rates and pitting corrosion damage due to biofilm formation on a stainless steel increased in the sequence: biofilm of iron-oxidizing microbes < SRB biofilm < biofilm of a consortium of iron-oxidizing microbes and SRB /XU 08/.

5.1 Evaluation of the safety relevance of the process "Microbially influenced corrosion and activity of sulphate-reducing bacteria"

The ability of SRB to accelerate corrosion of waste canister or over-pack materials in the reference designs of engineered barrier systems adopted in different countries (carbon steel in Belgium, France, Switzerland or copper in Sweden, Finland, Canada) is a major concern of microbial research related to final disposal of radioactive waste in geological formations /NTB 02/, /OND 04/, /AND 05/, /NWM 05/, /SKB 06/, /POS 07/, /NTB 09/. To deal with the concerns of the Swiss Federal Government over the corrosion issues in the Nagra' project "Entsorgungsnachweis", which had assessed the feasibility of HLW/SF disposal in a DGR in Opalinus Clay /NTB 02/, Nagra has convened the Canister Materials Review Board to consider the corrosion behaviour of HLW/SF canister materials suitable for the proposed repository environment. In the resulting report, the Board has recommended to abandon the efforts to demonstrate the inactivity of SRB in a DGR and to focus instead on evaluating the sustainability of the MIC and the maximum related damage /NTB 09/.

The presence and activity of SRB in Opalinus Clay and Boom Clay formations as well as in commercial Wyoming MX-80 bentonite proposed as a buffer material has been proved in a series of extensive studies /BOI 96/, /DEC 04/, /KUR 04/, /MAU 07/, /STR 07/, /CHI 08/, /AER 09a/, /AER 09b/, /LYD 10/, /MAS 10a/, /MAS 10b/, /PED 10/, /STR 10a/, /STR 10b/, /STR 11/. Their abundance in the studied clays could however not been reliably determined due to limitations of the quantitative experimental methods applied usually instead of the extremely tedious direct microscopic counting. Microbe enumeration methods relying on the microbe culturability[16] generally suffer from the intrinsic microbe species-specificity of culture media on the one hand, as well as the dissimilarity of culture conditions – with, e.g., temperature playing a crucial role – and media from the natural subsurface environment of microbes on the other hand /LIT 07/.

It is now widely accepted that ~99.9 % of microorganisms from the environment resist cultivation in the laboratory /SAR 05/, /AND 06/, /LIT 07/, /STR 10a/. Moreover, culturable microbes can enter into a non-culturable state upon a change of the environ-

[16] Culturability of microbes populating a sediment sample expresses their ability to experience a stimulated growth upon dispersing the sample in a nutrient-rich medium and incubating it at laboratory conditions for time periods of up to several months.

mental conditions /LIT 07/, and the extent of this adaptive response can be hardly predicted. For example, a decrease of culturable population of microbes by two to five orders of magnitude can parallel the decrease of viable population of the same microbes by only two to three times occurring upon an increase of dry clay density from 0.8 g cm^{-3} to ≥1.3 g cm^{-3} or upon a decrease of clay water content /STR 10a/, /STR 11/.

This renders presently available culturability methods rather inappropriate for quantitative comparisons of microbial populations in sediment samples taken at different locations (even of the same geological formation), unless it can be proved that the locations are characterized by the same local environmental conditions and the samples had the same treatment record. Considering these limitations, it is difficult to conclude on how representative of the true values are, e.g., the populations of at maximum ~10^4 SRB per g clay (compacted to a dry density of 1.7 g cm^{-3}) determined by a culturability method in bentonite-based buffer and backfill materials, which have been sampled after accomplishing 1.5 to 6.5 year-long full-scale placement experiments at a depth of 420 m in AECL's underground research laboratory /STR 10a/.

Similarly, quantitative polymerase chain reaction (Q-PCR) on DNA extracts from subsurface sediments, which is superior to culture methods in allowing detection of non-culturable microbes /BOI 96/, can provide results strongly contradicting to microscopic analyses /REI 11/. Despite the obvious SRB activity in the in situ borehole experiment in the Mont Terri underground research laboratory, the Q-PCR method did not show any presence of SRB in clay /STR 11/. Furthermore, no SRB species could be identified in the clay using a SRB enrichment medium, but a spore-forming SRB has been detected there using a methanogenic culture medium. Again, ~10^4 SRB per ml borehole solution and only ~10^3 SRB per g of wet clay have been measured with a culturability method at the end of the experiment. The higher SRB content in borehole solution as compared to that in clay – apart from being biased from the true values as discussed in the preceding paragraph – can be due to a dislodging of biofilm material from the clay surface upon removal of all the water from the borehole, as suggested by the authors of the study to explain that the total number of planktonic microbes at termination was two to three orders of magnitude higher than during the experiment /STR 11/.

The in situ studies carried out in underground research facilities and large-scale mock-up experiments have clearly demonstrated that decreased material density and in-

creased water availability in particular at the waste canister–clay buffer and clay buffer–host rock interfaces but also in the excavation damaged zone of a DGR in a deep claystone can form environments conducive to microbes /DEC 04/, /KUR 04/, /AER 09a/, /AER 09b/, /LYD 10/, /STR 11/, /WER 11a/. An estimation of the extent of the increase of microbe population related to such formation currently seems to be rather not possible, as the available scarce data – e.g., an observation of a factor of 10^5 higher number density of SRB at the steel–clay interface than 2 m farther from that in clay /AER 09a/ – have been obtained with culturability methods, which do not allow a reliable quantitative estimation as discussed in the preceding paragraphs. Still, the latter study has reported severe corrosion of the steel surface that – together with the indication of the favoured SRB growth – can be assumed to be an indication of MIC despite only trace amounts of detected sulphide.

This assumption is reasonable considering the above discussed ability of, e.g., iron-oxidizing microbes to induce MIC and to favour SRB growth or of SRB to grow by reducing Fe(III) instead of sulphate. In fact, SRB have been shown to prefer Fe(III) over sulphate as the terminal electron acceptor at low H_2 concentrations /COL 93/. Furthermore, many SRB species can grow fermentatively (see the next section for more details) or reduce nitrate without producing sulphide, so that the occurrence of high numbers of SRB in an environment has been concluded not necessarily to reflect the occurrence of sulphate reduction there /MUY 08/.

Another reason in support of this assumption is that microbial production of sulphide can be coupled to the reaction of the sulphide with elemental sulphur adsorbed at (or incorporated in) the steel surface, so that actually no or only trace amounts of sulphide can escape the cyclic process of MIC in this case. Indeed, as discussed above, sulphur adsorbed on an exposed metal surface is considered to be the critical species for pitting corrosion of metal /WIL 10/.

At the same time, one of the two pathways of microbial respiration of elemental sulphur is based on reduction of dissolved polysulphides (S_n^{2-}, $n > 2$), formed from S^0 at pH > 7, to hydrogen sulphide and the chemical reaction of the latter with S^0 to produce further polysulphide to sustain this microbial process /BUR 09/. In the second pathway of microbial respiration of S^0, thiosulphate ($S_2O_3^{2-}$) is reduced to yield hydrogen sulphide and sulphite (SO_3^{2-}), and the sulphite reacts chemically with extracellular S^0 to re-produce thiosulphate /BUR 09/. The latter study has demonstrated that Fe(III)-

reducing microbes can respire S^0 according to these pathways (in both cases producing hydrogen sulphide as long as the regeneration of that or thiosulphate is sustained by the corrosion reaction). Since many SRB species are able to respire S^0, similar effect can be expected for SRB populating the steel–clay interface.

The mock-up experiment OPHELIE, which has revealed the presence of corrosion of heated stainless steel in association with, most likely, ferrous sulphides /KUR 04/, further highlights the need for considering the two following possible scenarios for activation of MIC by SRB at the conditions of a DGR for HLW/SF. In the first scenario, according to the conditions of the experiment, SRB (or Fe(III)-reducing microbes able to reduce thiosulphate to hydrogen sulphide as discussed above) ought to be considered to be able to metabolize and produce sulphide at temperatures much above 100 °C (up to 137 °C) near the metal–sand interface[17]. The observed corrosion can in principle be caused by each of the four MIC mechanisms described at the beginning of the current section, and an identification of the responsible mechanism is not possible in this case, as the experiment has not been designed for this purpose.

In the second scenario, MIC ought to be considered to proceed by attack of hydrogen sulphide, which may not necessarily be produced within the backfill but can diffuse through it from a distant location more favourable for SRB activity (in the experiment, possibly from the hydration circuit). An alternative, non-MIC scenario of thermal sulphate reduction to sulphide has been discarded due to very slow reaction kinetics at the applied maximum temperature of ~140 °C /DEC 04/. Indeed, this thermochemical reaction (reduction of sulphate by aqueous acetate) has been found to only initiate in the presence of elemental sulphur and estimated to halve the aqueous sulphate concentration in ~1600 years at 150 °C /CRO 04/. The rate of this reaction decreases exponentially with temperature, so that the half-time of aqueous sulphate increases to ~20,000 years at 125 °C.

Although the first scenario obviously requires going beyond the common perception of what life is able for, the above discussed experimental studies provide evidences of ability of SRB not only to survive at such severe conditions but also to reduce sulphate at such high clay densities /CHI 08/ or pressures of 20 to 50 MPa /ROS 91b/, which theoretically correspond to even higher density values. Still, the present survey of the

[17] As mentioned above in the discussion for the Figure 5.3, the inner and middle rings of clay-based backfill blocks were replaced by sand and concrete, respectively, near the mock-up's main cover /VER 03/.

relevant literature could not find any evidence of the ability of SRB to produce sulphide when both temperature and clay density are so high. This does not exclude such a possibility, but simply reflects the current state of knowledge.

The second scenario is supported by the experimental study that has demonstrated and characterized hydrogen sulphide diffusion through a compacted water-saturated clay /PED 10/. However, that study has derived diffusion coefficients of 0.8 $cm^2\ yr^{-1}$ and 4.8 $cm^2\ yr^{-1}$ at the clay densities of 2.0 g cm^{-3} and 1.75 g cm^{-3}, respectively, and at the temperature of 22 °C. Applying the Stokes-Einstein relation between diffusion coefficients, viscosities, and temperatures /LI 74/ as well as the values of 1042 and 211 µPa s for viscosities of 0.5 mol kg^{-1} NaCl solutions at 20 °C and 140 °C, respectively /KES 81/, the hydrogen sulphide diffusion coefficients in the clay pore water of the OPHELIE experiment at the maximum temperature of 137 °C can be estimated to equal 5.5 $cm^2\ yr^{-1}$ and 32.9 $cm^2\ yr^{-1}$ at the clay densities of 2.0 g cm^{-3} and 1.75 g cm^{-3}, respectively. The latter diffusion coefficient would correspond to a hydrogen sulphide migration of at maximum only ~13 cm during the five-year experiment, which is much lower the distance than the thickness of the clay-based buffer layer[18]. Reasonably assuming that no advection migration path existed in the mock-up experiment setup, a factor of up to ~30 higher hydrogen sulphide diffusion coefficient (of up to ~1,000 $cm^2\ yr^{-1}$) ought to be postulated instead in order to reach agreement with the experimental observation. It can be thus concluded that the issues related to SRB activity at the conditions of a DGR for HLW/SF in clay remain unsatisfactorily understood.

During the diffusion through clay, hydrogen sulphide can react with structural Fe(III) reducing it and releasing Fe(II) ions to potentially influence safety-relevant properties of clay (see the discussion on the safety-relevant processes “Microbial reduction of clay minerals” and “Microbial dissolution of clay minerals”). In the in situ borehole experiment in Opalinus Clay, which was already discussed in detail above, the concentration of dissolved Fe(II) followed with some time lag a trend shown by hydrogen sulphide and showed an intermediate increase from ~2 µmol l^{-1} expected in the clay pore water to a rather high value of ~100 µmol l^{-1} /WER 11a/. Although the authors of the study have argued that this Fe(II) may have been released from Fe(II)-carbonate minerals (e.g., siderite $FeCO_3$) or desorbed from the clay, microbial reduction of Fe(III) and re-

[18] Note that the thickness of the clay-based buffer layer of 72.5 cm was reduced by a factor of ~3 (to ~24cm) above the corroded location on the heat-generating tube as a result of a partial replacement of the clay-based backfill by sand and concrete (see the preceding footnote).

duction by hydrogen sulphide should be accounted for as a possible source of Fe(II) either.

In fact, at least two subsurface microbial species have been shown to utilize insoluble Fe(III) as terminal electron acceptor solely via reduction of elemental sulphur to sulphide and the subsequent reduction of Fe(III) by the sulphide with the regeneration of elemental sulphur /HAV 08/. As discussed above, the release of Fe(II) upon Fe(III) reduction by SRB-produced sulphide can be about four times higher than for inorganic sulphide due to presumably SRB-produced extracellular polymers /LI 04/. Their favourable adsorption on clay surfaces can be suggested however to lead to a localization of this facilitating effect to the close vicinities of the active SRB, whereas clay interaction with hydrogen sulphide unaided by extracellular polymers will occur at more distant locations.

For the latter case, observations made in subsurface media can be used to estimate the outcome of clay reaction with hydrogen sulphide. Clay-containing sediments at depths of 1 m up to 80 m below the surface have been concluded to be characterized by clay-Fe(III) half-lives of 8.4×10^4 up to 2.4×10^6 years depending on the deposition rate and mineralogy of the sediment when exposed to a 1 mmol l^{-1} solution of SRB-produced hydrogen sulphide /CAN 92/, /RAI 96/. The former half-life value, which should be used in a conservative approach if no value specific to the clay host rock of a DGR is available, is significantly lower than the timeframe of one million years after repository closure assigned in accordance with regulatory requirements in many countries for the assessment of the safety of potential DGR for HLW/SF. This underlines the necessity of a quantification of the maximum potential effect of SRB activity in a DGR not only for canister corrosion but also for clay buffer and clay host rock, especially considering that sulphide concentrations of 0.5 and 1.0 mmol l^{-1} have been observed in the groundwater and borehole solution at the Äspö and Mont Terri underground research laboratories, respectively /MAS 10a/, /WER 11a/.

Sulphate required for SRB activity is available in pore water of Opalinus, Callovo-Oxfordian clay formations at concentrations of ~15 mmol l^{-1} /ELH 10/, /WER 11a/. In undisturbed Boom Clay, sulphate concentration does not generally exceed ~0.1 mmol l^{-1} but has been found to increase from ~2 mmol l^{-1} several meters from an excavation gallery to ~60 mmol l^{-1} near the gallery wall due to oxidation of pyrite (FeS_2) /AER 09b/. This process has been concluded to be responsible for sulphate production

in Boom Clay, which contains 1 - 5 weight % of pyrite, within about one meter from the clay interface with the concrete lining of the HADES tunnel /DEC 08/. As a result of the pyrite oxidation, Fe(II) is released as well at a concentration which is two times lower than that of the released sulphate. The latter work argues that in the Boom Clay pore water, Fe(II) dominates aqueous iron speciation. Accordingly, the sulphide concentrations of up to 5.6 mmol l^{-1} measured in the pore water near the lining of the HADES tunnel /LYD 10/ can even underrepresent the actual sulphide production by SRB. Indeed, Fe(II) diffusion along with sulphate from the oxidized zone near the tunnel lining into the clay pore spaces populated with active SRB would result in precipitation of ferrous sulphides as observed in the in situ experiment carried out in the Opalinus Clay in the Mont Terri underground research laboratory /STR 11/, /WER 11a/.

Furthermore, clay buffer around radioactive waste canisters can become exposed to sulphate concentrations above 40 mmol l^{-1} characteristic to granitic groundwater at a depth of a potential DGR in Sweden /MAS 10a/. Alternatively, although sulphate concentration in granitic groundwater at the AECL's underground research laboratory in Canada equals ~0.5 mmol l^{-1} /BRO 94/, it can increase up to ~10 mmol l^{-1} in the clay buffer due to dissolution of such accessory minerals of clay as gypsum /STR 10a/. In the latter case, only less than 0.5 % of initially present sulphate has been converted to sulphide due to SRB activity during a 6.5-year-long in situ experiment. However, this rather low reduction rate may be attributed to oxic conditions – which are not conducive to SRB – as less than 10 % of O_2 were reduced in the clay buffer in this period /STR 10a/. Last but not least, oxidation of sulphide to sulphate by O_2, H_2O_2, and OH radicals, which has been suggested to explain increased sulphate concentrations in the deep pore waters, produced radiolytically at the interface with the waste canisters can provide a cycling supply of sulphate sustaining SRB activity /LIN 05/.

It is noteworthy that some products of SRB activity can impede that activity. A strong inhibition of SRB activity has been observed in laboratory experiments with Boom Clay pore water at sulphide concentrations of 13.5 mmol l^{-1}, whereas 5.6 mmol l^{-1} of sulphide were able to inhibit SRB activity in some cases /AER 09c/. Yet in another study with Boom Clay as high sulphide concentrations as of 36 mmol l^{-1} have been measured as a result of the activity by SRB enriched from Boom Clay pore water and mixed with the Boom Clay sample /LYD 10/. In the case of sustained steel corrosion, however, no such high sulphide concentrations can be expected due to the formation of ferrous sulphides consuming dissolved sulphide. Another product of SRB activity in clay

can be aluminium released as a result of microbial reduction of Fe(III) and subsequent clay mineral dissolution as discussed for the safety-relevant process “Microbial dissolution of clay minerals”. Dissolved aluminium concentrations in excess of 50 µmol l^{-1} have been found to substantially inhibit growth of SRB population /AMO 03/. However, pitting corrosion rates of up to ~500 µm yr^{-1} have been observed for corrosion of stainless steel influenced by unidentified microbes remaining active at dissolved aluminium concentrations of ~100 µmol l^{-1} /WOL 97/.

In its report to Nagra, the Canister Materials Review Board postulates that it is reasonable to assume that corrosion of steel canisters in saturated bentonite is uniform, so that the corroded area equals the canister surface /NTB 09/. However, recent studies of SRB-induced MIC in clay environment /KUR 04/, /ELH 10/ suggest that such an assumption cannot be generally considered valid, as can be also seen in Figs. 5.2 A and 5.3 B. On the contrary, according to these two examples, a localized, pitting corrosion rate of ~30 up to ~600 - 700 µm yr^{-1} should be assumed for MIC of steel in contact with clay instead. The latter value corresponds well to the maximum corrosion rate as a result of MIC at anaerobic conditions reported elsewhere /SHE 11/ for non-clay environments as discussed at the beginning of the current section. Therefore, the demonstrated ability of SRB to induce extensive pitting corrosion damage even at low levels of biomass covering a minute fraction of the steel surface /SHE 11/ has to be taken into account when evaluating possible MIC effects in a DGR for HLW/SF.

It cannot currently be concluded from the available experimental data whether, and if so to what extent, the pitting corrosion is favoured by each of the identified MIC mechanisms. The local character of this corrosion kind indicates, however, that a direct contact between SRB or their extracellular polymers and the corroding surface as required in the MIC mechanisms (b), (c), and (d) discussed at the beginning of the current section may be especially favourable for it. Alternatively, though, the pitting corrosion may result from local material defects, in which case all MIC mechanisms should be accounted for in order to elucidate the major mechanism responsible for it. Clearly, more research should be done on this topic in order to be able to evaluate the possibility of occurrence of pitting corrosion in a DGR for HLW/SF. Furthermore, in no case should the potential impact of SRB be underestimated based on a possible argument of comparably low biomass of the microbes in contact with metal surfaces or dissolved metals. The timeframe of their potential activity must be considered concomitantly, as in a space of several years SRB encountering sub-micrometre concentrations of dissolved

zinc are able to deposit a zinc-sulphide-rich biofilm that if continued over geologic time would produce an economic deposit /MAC 07/.

5.2 Summary of the specifications of the process "Microbially influenced corrosion and activity of sulphate-reducing bacteria"

Safety-relevant properties of clay influenced[19]:

(i) swelling pressure (negatively influenced),
(ii) specific surface area (negatively influenced),
(iii) cation exchange capacity (negatively influenced),
(iv) anion sorption capacity (negatively influenced),
(v) porosity (negatively influenced),
(vi) permeability (negatively influenced),
(vii) fluid pressure (negatively influenced),
(viii) plasticity (negatively influenced).

Barriers with safety-relevant containment function affected:

(i) waste canisters,
(ii) clay buffer,
(iii) claystone.

Mechanisms:

(i) hydrogen sulphide attack,
(ii) adsorption of microbially produced extracellular polymers at the metal–water interface,
(iii) scavenging of hydrogen atoms from the metal surface,
(iv) direct uptake of metal electrons into microbial cells.

[19] Since interaction of SRB-produced hydrogen sulphide with clay leads to clay dissolution, the safety-relevant properties identified for the safety-relevant process "Microbial dissolution of clay minerals" are adopted here.

Reactions involved[20] (a relevant selection):

Reduction of aqueous SO_4^{2-} coupled to oxidation of hydrogen /THA 77/

$$SO_4^{2-} + 4\ H_2 + H^+ \rightarrow HS^- + 4\ H_2O, \qquad (5.1)$$

with $\Delta G°' = -152$ kJ/reaction,

acetate /MUY 07/

$$SO_4^{2-} + CH_3COO^- \rightarrow HS^- + 2\ HCO_3^-, \qquad (5.6)$$

with $\Delta G°' = -48$ kJ/reaction,

or lactate /MUY 07/

$$0.5\ SO_4^{2-} + CH_3CHOHCOO^- \rightarrow 0.5\ HS^- + CH_3COO^- + HCO_3^-, \qquad (5.3)$$

with $\Delta G°' = -80$ kJ/reaction.

[20] Free energy increments $\Delta G°'$ are given according to /THA 77/ and /MUY 08/ for reactions under standard conditions – i.e. 25 °C, 0.1 MPa, water activity of 1, solute activities of 1 mol kg^{-1} – except that physiological pH value of 7 is taken instead of the standard activity of 1 mol kg^{-1} for H^+, which corresponds to pH value of 0.

6 Microbial gas production and conversion

It is commonly recognized that H_2 produced as a result of corrosion of iron is the gas of the most relevance to assessing the integrity of technical barriers and host rock for DGR for HLW/SF in clays /NOS 12/. The latter work points out that waste management organisations have increasingly shifted their focus to DGR design as a part of the strategy to diminish the potential impact of gas generation and transport. Thereby, the major concern is with a build-up of overpressure to levels that would result in fracturing of clay, which could result in an increased release of volatile radionuclides – mainly ^{14}C and, possibly, ^{129}I – and their transport via the carrier gas H_2. In a further consequence, gas generation can cause changes in geochemical conditions with, e.g., dissolved CO_2 representing a strong ligand facilitating the transport of actinides /NOS 12/.

The microbial reduction of Fe(III) or sulphate considered in the preceding sections leads to production and/or consumption of gases. When Fe(III)-reducing microbes utilize organic compounds (see a detailed discussion on electron-acceptor limited subsurface settings in the next section) to gain energy during the reduction process, one mole of CO_2 is produced per one mole of carbon atoms of the oxidized organics and four moles of the reduced Fe(III) (see the summary of the specifications of the safety-relevant process “Microbial reduction of clay minerals”). In the absence or limited supply of organics, on the contrary, one mole of H_2 is necessary for microbes to reduce two moles of Fe(III).

Similarly, one mole of gaseous hydrogen sulphide (H_2S) and two moles of CO_2 are produced by SRB per one mole of reduced sulphate with acetate as electron donor, but four moles of H_2 must be microbially consumed to produce one mole of H_2S if only H_2 is available as electron donor (see the summary of the specifications of the process “Microbially influenced corrosion and activity of sulphate-reducing bacteria”). A reaction of one mole H_2S with dissolved or insoluble Fe(II) or Fe(III) can result in a release of one mole H_2, whereas a reaction of four moles of atomic hydrogen with carbon steel can lead to a decarburization of the latter and the formation of one mole of methane (CH_4).

This section proceeds by considering three further microbial processes of high importance in the deep subsurface – fermentation, anaerobic methane production and anaerobic methane oxidation – which can potentially contribute to net production (H_2, CH_4, CO_2, H_2S) or consumption (H_2, CH_4, CO_2) of gases and be responsible for gas conversions (e.g., conversion of CO_2 to CH_4) in a DGR for HLW/SF.

6.1 Fermentation

Fermentation refers to microbial metabolic processes that do not employ external electron acceptors for oxidation of organics and result in the accumulation of reduced compounds such as acetate (CH_3COO^-), formate ($CHOO^-$), and H_2 as well as oxidized carbon (CO_2) /MCM 92/. In this process, up to two moles of H_2 and up to one mole of CO_2 are produced per mole of carbon atoms of the oxidized organics, as e.g., for glucose /THA 77/:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (6.1)$$

or

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2. \quad (6.2)$$

Some microbial species, which often co-exist with SRB species requiring acetate as carbon source, are also able to metabolize these gaseous products in a reverse reaction consuming four moles of H_2 and two moles of CO_2 per one mole of produced acetate:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O. \quad (6.3)$$

Fermentation has been shown to occur at geologically significant rates in buried sediments of the Black Creek formation in the Atlantic Coastal Plain of South Caroline, USA /MCM 92/, /CHA 96a/. These sediments are characterized by clay layers with a thickness of up to ~10 m and organic carbon content of up to ~0.7 weight % interbedded with sands at depths of ~50 up to ~900 m. As a result of fermentation, a net diffusive flux of low molecular organic acids and CO_2 from clay into sands has been occurring during the post-depositional period, which favored activity of SRB at clay–sand interfaces. As high acetate and formate concentrations as of 1.8 and 6.4 mmol l^{-1}, measured in clay pore waters at a depth of 830 m, were decreased by the SRB activity to values below 2×10^{-3} mmol l^{-1} in the adjacent sand pore waters. This activity led to an increase of the flux of dissolved inorganic carbon into sands in addition to that due to a reaction of fermentatively produced CO_2 with carbonate minerals in clay /CHA 00/:

$$CaCO_3 + CO_2 + H_2O \leftrightarrows Ca^{2+} + 2HCO_3^-. \quad (6.4)$$

Accordingly, the net flux of CO_2 of estimated 3×10^{-5} mmol l^{-1} yr^{-1} from clay into sands was accompanied by a three times smaller CO_2 flux from SRB activity and has been

concluded to be responsible for at least ~10 % of the carbonate carbon accumulated in Black Creek sands since their deposition ~70 million years ago /MCM 92/.

6.2 Anaerobic methane production

Methane production (methanogenesis) by strictly anaerobic microbes occurs by the three following major pathways through (a) reduction of CO_2, (b) fermentation of acetate, and (c) fermentation of methanol or methylamines.

(a) Reduction of CO_2 with electrons derived from oxidation of H_2, formate or CO:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O, \quad (6.5)$$

$$4HCO_2H \rightarrow CH_4 + 3CO_2 + 2H_2O, \quad (6.6)$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2. \quad (6.7)$$

(b) Fermentation of acetate, in which COOH group is oxidized to CO_2 to provide electrons for reduction of the methyl group to methane:

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2. \quad (6.8)$$

(c) Fermentation of methanol or methylamines, in which electrons derived from oxidation of either a methyl group (to CO_2) or H_2 (for at least one microbe species) are used for reduction of the methyl group(s) to methane, as e.g. for methanol /THA 08/, /FER 10/:

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O, \quad (6.9)$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O. \quad (6.10)$$

Whereas the first pathway can result either in a fourfold reduction of gas content (CO_2 being easily soluble and converted into HCO_3^- at pH values relevant for clay pore waters) or its net increase in the pore water or in a gas conversion, the second one leads to a net increase of gas content only, and in the third one either a net increase of gas content or a gas conversion occurs. It is estimated that the two-thirds of CH_4 exhaled into the atmosphere by Earth's biosphere is produced from acetate according to the second pathway, whereas the remaining methane mostly stems from CO_2 reduction following the first pathway /FER 10/.

However, these global relative contributions from the methane production pathways do not necessarily apply to the deep subsurface. Extensive core samplings of deep (down to ~300 m below seafloor) marine sediments off Vancouver Island at the western coast of Canada have recently revealed that methane production by CO_2 reduction pathway was by one to three orders of magnitude larger than that by acetate fermentation /YOS 10/. This ratio has been observed along the whole depth profile in the constituting sand, silty clay, and clay layers with methane production rates reaching in the latter up to a few 10^{-6} mmol l^{-1} day^{-1}. The net methane production in these sediments is possibly underestimated by up to a factor of ten because of anaerobic methane consumption by methane-oxidizing microbes (see a detailed discussion on this process below) co-populating some of the sediment layers. This deep subsurface methane production has been concluded to probably contribute to methane hydrate formation and accumulation in the one of the largest deposits of gas hydrate, which is restricted to the depth interval of ~130 - 220 m and hosted in silty clay below the depth of ~170 m.

Similarly, in a petroleum-hydrocarbon contaminated shallow aquifer in Studen, Switzerland, methane production by CO_2 reduction pathway was characterized by about ten and twenty times higher rates compared to those for acetate and methanol fermentations, respectively /KLE 05/. However, in this case, the population of acetate-fermenting methanogens was 15 - 25 times larger than that of CO_2-reducing methanogens, which has been interpreted as an indication of increased availability and methanogenic role of acetate in this aquifer. Though active in the studied groundwater and sediments, methanol reducers were producing methane at twofold lower rates than acetate-fermenting methanogens and have been concluded to make only a minor contribution to the overall methane production.

Furthermore, CO_2-reducing methanogens have been inferred to play a major role in methanogenesis occurring ~100 m below land surface in an underground oil storage cavity in Japan /WAT 02/. The methanogenesis was at least fivefold more intensive than other microbial reduction processes and resulted in a production of ~0.14 mmol l^{-1} day^{-1} of methane. The latter study has pointed out that the importance of acetate-fermenting methanogens – when assessed by the DNA cloning and sequencing analysis – may have been overestimated in studies of anaerobic hydrocarbon degradation. Instead a discrimination based on production rates characteristic to different groups of methanogens has been advocated to avoid possible misinterpretations. Indeed, a specific methane production rate of ~1.92 mmol l^{-1} per mg of dry cells was

measured in the environmental sample from the oil storage cavity using endogenous substrate. As documented in the latter study, this rate is comparable to the value of 1.4 mmol l^{-1} per mg of dry cells characteristic of CO_2-reducing methanogens but is much higher than that of 0.05 mmol l^{-1} per mg of dry cells reported for acetate-fermenting methanogens.

In contrast to these three examples of subsurface methanogen populations, however, an anaerobic biofilm sampled in a borehole ~1.5 km below land surface exhibited the clear prevalence of acetate-fermenting methanogens as expected from the global atmospheric methane release /MAC 07/. Similarly, acetate fermentation was the predominant pathway of methanogenesis in a shale aquifer contaminated with gas condensate /STR 05/. In line with these contrasting observations, two neighbouring wells monitoring formation waters from the deep repository of liquid radioactive wastes in a sand-clay rock formation near Zheleznogorsk, Russia, have revealed alternating prevalence of CO_2 reduction (by ~300 times, at a depth of 170 m) or acetate fermentation (by ~3 times, at a depth of ~400 m) pathway of methane production /NAZ 04/. This study has reported the maximum rate of methane production of 6.8×10^{-6} mmol l^{-1} day^{-1} due to CO_2 reduction as compared to that of 1.6×10^{-6} mmol l^{-1} day^{-1} due to acetate fermentation.

In the in situ experiment carried out in the Opalinus Clay in the Mont Terri underground research laboratory, methane concentration has been observed to steadily increase for about four years and to reach a value of 0.7 mmol l^{-1}, which was by three orders of magnitude higher than that at the start of the experiment /WER 11a/. Direct detection of methanogens in the borehole water and the contacting clay as well as isotopic analyses of released methane confirmed that its production resulted from microbial methanogenic activity /STR 11/. Though that experiment featured a setting where methanogenesis has been concluded – based on a reactive transport modelling effort – to play only a minor role in degradation of the available organic carbon /WER 11b/, the observed pore water methane concentration is comparable to that of up to ~1.1 mmol l^{-1} in the groundwater of a shallow sandy aquifer contaminated by gas condensate in Colorado, USA, where methanogens were the major microbial species consuming acetate /GIE 99/.

Furthermore, the methane concentration measured in the Opalinus Clay borehole is only about two times lower than that of up to ~1.6 mmol l^{-1} measured in a shallow

sandy aquifer contaminated by crude oil in Minnesota, USA, where methanogenesis has been identified as a major process /BAE 93/. A very similar methane concentration of ~0.7 mmol l^{-1} has been also observed in the shallow aquifer contaminated by petroleum hydrocarbons /KLE 05/. The authors have argued that degradation of the latter compounds may be contributed considerably by methanogenesis both (i) indirectly, by keeping H_2 concentrations low enough for fermentative microbes to grow, and (ii) directly, by cleaving end products of fermentations (acetate, methanol). Thus, sediments that are characterized by sufficient availability of electron donors or depleted of terminal electron acceptors other than CO_2 (primarily, Fe(III) or sulphate) can generally represent environments favourable to methanogens (see section “Electron-acceptor limited subsurface settings” for further discussion related to the competition between microbial reduction processes).

Inhibition of methane production in the presence of Fe(III) had been initially attributed to the ability of Fe(III)-reducing microbes to outcompete methanogens for acetate and hydrogen, their two primary electron donors /LOV 87/, /HUR 97/, /CHA 00/. However, further studies have revealed an additional mechanism of the inhibition. Methanogens producing methane by the three major pathways have been found to be able to reduce solid-state Fe(III) as well as extracellular quinones /BON 02/, which can serve as electron shuttles to promote reduction of solid-state Fe(III) as discussed above for the safety-relevant process “Microbial reduction of clay minerals”. Some species simultaneously reduced Fe(III) and produced methane (at decreased levels) while exhibiting population growth, whereas other produced no methane and showed no growth while reducing Fe(III). Furthermore, methanogens, which had been considered for a long time to be strict anaerobes requiring a low redox potential to be active, were able to rapidly initiate methanogenesis in suspensions containing oxidized humic acids or Fe(III) oxides /BON 02/. With acetate as the sole electron donor though, no reduction of solid-state Fe(III) has been observed in the latter study.

This observation has been confirmed in a recent study, which demonstrated that methanogens can reduce structural Fe(III) in iron-rich smectites with H_2 and methanol as electron donors, but not with acetate /LIU 11/. The extent of Fe(III) reduction by methanogens (~1.4 mmol g^{-1} clay within four weeks) was very similar to that observed for the same smectite exposed to a typical Fe(III)-reducing microbial species /JAI 07b/. In a further accordance with the observations made for Fe(III)-reducing microbes, the reduction of structural Fe(III) in clay to this extent led to a partial dissolution of the

smectite (see also corresponding discussion for the safety-relevant processes “Microbial reduction of clay minerals” and “Microbial dissolution of clay minerals”).

In extension of their ability to reduce solid-state Fe(III), methanogens have been recently revealed to produce methane and hydrogen necessary for its production during the studied time period of about five months on a mixture of 1 g of metallic iron and 1 g of montmorillonite suspended in 5 ml of bicarbonate buffer /CHA 10/. Although no mechanism explaining this observation has been proposed, the lacking production of H_2 and CH_4 in sterile controls or controls without metallic iron do represent a strong evidence for the ability of methanogens to facilitate corrosion of iron. The observation of by at least three orders of magnitude higher methane production and corrosion rates in a mixture of a fine powder of metallic iron with Boom Clay–water slurry as compared to a mixture containing steel instead of metallic iron /ORT 02/ can be considered as a further demonstration for this methanogens’ ability. Incubation of marine sediments collected near Wilhelmshaven, North Sea, with iron granules in another study has demonstrated that methanogens can obtain electrons from metallic iron at rates which are more than one order of magnitude higher than those expected from a mere consumption of H_2 formed on metallic iron surface by water attack (see discussion for the safety-relevant process “Microbially influenced corrosion and activity of sulphate-reducing bacteria” for more details on the latter corrosion mechanism) /DIN 04/. The mechanism by which methanogens utilize metallic iron as electron donor remains still unknown.

Besides the rather diversified mechanisms of gaining energy for metabolism and growth, methanogens can also exhibit extraordinary survival capabilities. The most heat-resistant microbe currently known is a methanogen isolated from a deep-sea hydrothermal habitat from a depth of ~2,400 m (Fig. 6.1) /TAK 08/. This methanogen can grow and produce methane at 122 °C under the in situ pressure of 20 MPa, whereas the maximum temperature of its proliferation decreases to 116 °C at 0.4 MPa. The latter observation confirms the emerging notion that the microbial response to the elevated hydrostatic pressure can strongly affect temperature ranges of growth and metabolic and biochemical functions of microbial cells.

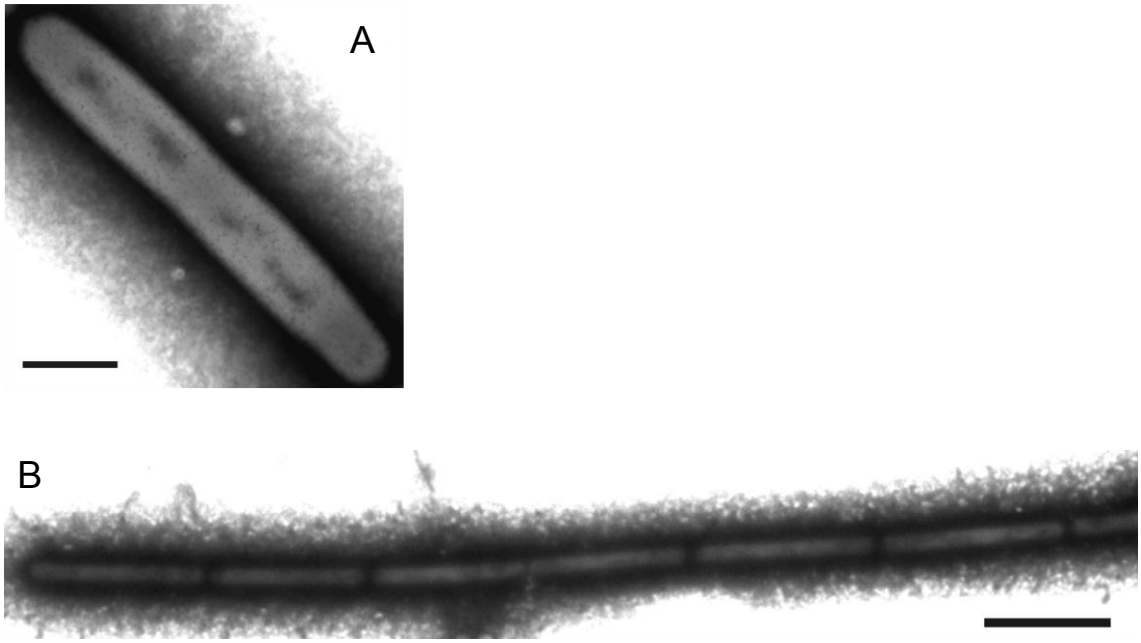

Fig. 6.1 Electron microscopy micrographs of (A) a hyperthermophilic methanogen growing and producing methane at 122 °C (scale bar corresponds to 1 µm) and (B) a typical filamentous association of the methanogen (scale bar corresponds to 5 µm) /TAK 07/

Yet the most important finding of the study may be the observation that stable isotope fractionation of methane carbon strongly decreases under high hydrostatic pressures to the values which had been previously attributed exclusively to the abiogenic, magmatic methane. The authors have suggested further that increased concentration of dissolved H_2 under a high hydrostatic pressure (estimated to be ~2.3 mmol l^{-1} at 0.4 MPa and ~120 mmol l^{-1} at 40 MPa) are responsible for the increased temperature ranges of growth and the decreased stable carbon isotope fractionation for this methanogen utilizing H_2 as electron donor.

6.3 Anaerobic methane oxidation

The anaerobic methanotrophic microbes gain energy by utilizing methane as electron donor and sulphate, nitrate, nitrite, Mn(IV) or Fe(III) as terminal electron acceptors to convert methane into carbon dioxide as shown below, e.g., for sulphate (note that one mole of hydrogen sulphide per mole of consumed sulphate is produced additionally in this case) and iron /KNI 09/, /BEA 09/:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O, \qquad (6.11)$$

$$CH_4 + 8Fe(OH)_3 + 15H^+ \rightarrow HCO_3^- + 8Fe^{2+} + 21H_2O. \quad (6.12)$$

The latter study has found that the energy gain by the anaerobic methanotrophic microbes in sediments almost doubles when Fe(III) is utilized as electron acceptor instead of sulphate despite the related about tenfold decrease in the methane oxidation rate from ~140 × 10^{-6} to ~16 × 10^{-6} mmol cm^{-3} day^{-1}. Although these methane oxidation rates may appear rather low, most of the methane produced below the seafloor is oxidized by the anaerobic microbes in the transition zones between sulphate-rich and methane-rich pore waters. Moreover, anaerobic methane oxidation is considered to possibly be the dominant sink for sulphate in marine sediments /DHO 02/.

The experimental study of marine sediments off Vancouver Island, which was discussed in the preceding subsection, has revealed that anaerobic methane oxidation in clay layers at depths of up to ~180 m below seafloor can exhibit rates of up to 10^{-8} mmol cm^{-3} day^{-1}, which presumably diminish the values measured for rates of concomitant methane production to the observed ones of up to a few 10^{-9} mmol cm^{-3} day^{-1} /YOS 10/. Furthermore, it has been suggested from isotopic evidence that methane oxidation reactions occurred along with methanogenic reactions in the borehole solution during the in situ experiment in the Opalinus Clay /WER 11a/.

Although the reaction mechanism and the identity of microorganisms responsible for anaerobic methane oxidation have not been firmly established yet, this process is most commonly attributed to the activity of consortia consisting of SRB and of microbes related to methanogens /KNI 09/. Such consortia have been found to remain active at temperatures of up to 95 °C in the hydrothermal sediments. However, anaerobic methane oxidation has been estimated to contribute only up to 5 % to the total sulphate reduction observed at higher temperatures in subsurface sediments /KAL 04/. Preliminary estimates suggest further that this process becomes much less important when sulphate concentration decreases below ~0.5 mmol l^{-1} /KNI 09/.

6.4 Evaluation of the safety relevance of the process “Microbial gas production and conversion”

A build-up of gas overpressure around a DGR for HLW/SF because of a disparity between the gas diffusion and gas production rates is considered to be a possible scenario which can result in a fracturing of the clay rock /ORT 02/, /NTB 02/. If occurs, this

fracturing, being preceded by the loss of clay plasticity (**safety-relevant property**), will be accompanied by gas discharge to relieve the excess pressure and, as such, will represent a temporal – due to self-healing processes in the presence of water – loss of the **safety-relevant containment function** of the clay. Importantly, during the build-up of gas overpressure, a DGR in clay populated with methanogenic microbes may represent a system characterized by a positive feedback with respect to the increase of hydrostatic pressure. This is because the increase can lead, on the one hand, to the accelerated growth and metabolic activity of microbial population – as discussed for Fig. **6.1** for the methanogen thriving at 122 °C – possibly resulting in a net gas production. On the other hand, it can lead to the formation of microfractures in clay well in advance of the gas breakthrough. Such microfractures, in turn, can be considered to represent environments conducive to microbes in addition to those at canister–clay buffer and clay buffer–host rock interfaces because of the associated decreased material density /DEC 04/, /KUR 04/, /AER 09a/, /AER 09b/, /STR 11/, /WER 11a/. Thus, the increase of hydrostatic pressure can facilitate the microbial activity which – provided it results in a net gas production – can contribute to a further increase of the gas pressure with the formation of microfractures making the increase potentially even stronger.

If the increased microbial activity leads to a net gas consumption as in the case of CO_2-reducing methanogens utilizing H_2 as electron donor (see the subsection “Anaerobic methane production” above), a system characterized by a negative feedback may result instead, in which an increase of hydrostatic pressure can be compensated by microbial gas consumption. However, a very recent evidence suggests that microbes can induce significant H_2 formation upon an addition of a range of common minerals and rocks to microbially populated sediment slurries at temperatures of 40 up to 100 °C with an optimum above 70 °C /PAR 11/. This addition resulted in an increase of the released amount of H_2 by up to 325 times and of CO_2 by about two times. Therefore, a microbial community consisting of microbes inducing such H_2 formation and of H_2-consuming methanogens may potentially be characterized – even though for only a limited period of time – by a net gas production and its habitat accordingly be representing a system with a positive feedback with respect to the overpressure build-up. It is therefore essential that a future quantitative estimation of the maximum possible effects of microbial processes in a DGR gives proper attention to a possible interplay of single microbial processes and not only quantifies their separate contributions.

The mechanism behind the observation of the significant H_2 formation in the study /PAR 11/ is currently unknown. The same applies to a further observation of the study that the foregoing microbial activity also enhanced abiotic gas-generating reactions occurring at higher temperatures of up to 155 °C. These observations illustrate that exogenous mineral- or rock-based materials placed in the microbially populated clay host rock of a DGR may represent an additional source of gas in the repository in general and of electron donors and acceptors for microbial activity in particular.

Considering the generally high heat-resistance of methanogens, increased temperatures in the vicinity of waste canisters in a DGR in clay can be assumed to not necessarily inhibit the methanogenic activity. In fact, thermophilic and hyperthermophilic methanogens can populate moderate environments, as substantiated by the enrichment of such species at 60 and 80 °C from the Opalinus Clay samples following the in situ borehole experiment characterized by pore water temperatures of 14 - 25 °C /STR 11/. Since the latter experiment has presented an evidence of the activity in the borehole solution of methane-oxidizing microbes, which can potentially remain sustained up to a temperature of 95 °C, methane oxidation should be accounted for as a relevant microbial process for a DGR in clay as well. Similarly, microbes responsible for fermentative production of H_2 and CO_2 from glucose or for consumption of H_2 and CO_2 to produce acetate have been found to survive and actively metabolize in sludge samples after a heat-treatment for two hours at a temperature of 104 °C /OH 03/. For a comparison, this same treatment has led to a complete inhibition of methanogenesis in the samples.

This example is in contrast to the observations of heat-resistant methanogens in the deep marine biosphere. However, the strong distinction between the deep terrestrial and deep marine habitats cannot be used to exclude the potential occurrence of a microbial species in both. Such an occurrence has actually been demonstrated for a hyperthermophilic fermentative and methanogenic microbe species populating both marine hydrothermal vent systems and fissure waters sampled at a depth of ~3 km below land surface in a deep gold mine /TAK 01/, /MOS 05/. It appears then that no definite conclusion about the potential presence or absence of methanogenesis in a DGR can be made based alone on the expected temperature development in the vicinity of waste canisters. Therefore, if no detailed information on the diversity of methanogenic, fermentative or methane-oxidizing microbial species in a DGR will be available, conservative estimations concerning their heat-resistance should be made. In accordance

with the available experimental evidence obtained for a moderate temperature range of 4 - 25 °C /KOT 07/, it should additionally be conservatively assumed that rates of microbial methane production increase with temperature, although some maximum temperature compatible with the rate increase may be derived from biological constrains on activity of methanogens.

6.5 Summary of the specifications of the process "Microbial gas production and conversion"

Safety-relevant properties of clay influenced:

(i) plasticity.

Barriers with safety-relevant containment function affected:

(i) clay buffer,
(ii) claystone.

Mechanisms:

(i) metabolism of gaseous, dissolved or solid organics and of gaseous or dissolved inorganic carbon.

Reactions involved[21] (a relevant selection):

1. Fermentation

Fermentative consumption of glucose /THA 77/

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2, \qquad (6.13)$$

with $\Delta G°' = -136$ kJ/reaction, or

$$C_6H_{12}O_6 + 6\ H_2O \rightarrow 6\ CO_2 + 12\ H_2, \qquad (6.14)$$

with $\Delta G°' = -26$ kJ/reaction,

[21] Free energy increments $\Delta G°'$ are given according to /THA 77/ and /MUY 08/ for reactions under standard conditions – i.e. 25 °C, 0.1 MPa, water activity of 1, solute activities of 1 mol kg^{-1} – except that physiological pH value of 7 is taken instead of the standard activity of 1 mol kg^{-1} for H^+, which corresponds to pH value of 0.

or production of acetate /THA 77/

$$4\ H_2 + 2\ CO_2 \rightarrow CH_3COOH + 2\ H_2O, \qquad (6.15)$$

with $\Delta G^{\circ\prime} = -95$ kJ/reaction.

2. Methane production

Following the first production pathway as discussed above by reduction of CO_2 with electrons derived from H_2 /FER 10/

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O, \qquad (6.16)$$

with $\Delta G^{\circ\prime} = -131$ kJ/reaction,

formate /FER 10/

$$4\ HCO_2H \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O, \qquad (6.17)$$

with $\Delta G^{\circ\prime} = -304$ kJ/reaction,

or CO /FER 10/

$$4\ CO + 2\ H_2O \rightarrow CH_4 + 3\ CO_2, \qquad (6.18)$$

with $\Delta G^{\circ\prime} = -211$ kJ/reaction.

Following the second production pathway by fermentation of acetate /FER 10/

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2, \qquad (6.19)$$

with $\Delta G^{\circ\prime} = -36$ kJ/reaction,

or methanol

$$4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2\ H_2O, \qquad (6.20)$$

with $\Delta G^{\circ\prime} = -319$ kJ/reaction.

Following the third production pathway by reduction of methanol with H_2 /THA 08/

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O, \qquad (6.21)$$

with $\Delta G^{\circ\prime} = -113$ kJ/reaction.

3. Methane oxidation

Methane oxidation with dissolved sulphate

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O, \quad (6.22)$$

with $\Delta G°' = -17$ kJ/reaction (ΔG of -14 kJ mol^{-1} has been reported for in situ conditions /BEA 09/),

or solid-state Fe(III) hydroxide

$$CH_4 + 8\ Fe(OH)_3 + 15\ H^+ \rightarrow HCO_3^- + 8\ Fe^{2+} + 21\ H_2O, \quad (6.23)$$

with $\Delta G°' = -92$ kJ/reaction (ΔG of -270 kJ mol^{-1} has been reported for in situ conditions /BEA 09/),

utilized as terminal electron acceptors /BEA 09/.

7 Electron-acceptor limited subsurface settings

In the late 1980s and early 1990s, growing experimental evidence has resulted in a formulation of criteria allowing prediction of the sequence in which microbial reduction processes will occur along the direction of groundwater flow in a pristine or contaminated subsurface environment based solely on information about the availability of key terminal electron acceptors and electron donors /LOV 87/, /LOV 88/, /LOV 94/, /CHA 96b/, /CHA 00/. It became increasingly clear that, e.g., the dominance order of microbial reduction processes characteristic to a pristine subsurface environment with a limited supply of electron donors (Fig. 7.1 A) can be reversed upon chemical contamination by human activities. As a consequence of an oversupply of electron donors, electron acceptors become rapidly – within years, as documented for a shallow aquifer by /BAE 93/ – exhausted near the contaminant source in the order O_2, NO_3^-, Fe(III), SO_4^{2-}, so that the local reversal can occur as schematically shown in Fig. 7.1 B.

These studies have also advocated the use of dissolved H_2 concentrations as an important aid in discriminating between zones of predominance of different microbial reduction process owing to the role of H_2 as a source of electrons for Fe(III)-reducing microbes, SRB, and methanogens (see corresponding reactions in the summaries of the specifications of the safety-relevant processes “Microbial reduction of clay minerals”, “Microbially influenced corrosion”, and “Microbial gas production”). In particular, Fe(III)-reducing microbes have been observed to maintain steady-state H_2 concentrations of 0.1 - 0.8 × 10^{-6} mmol l^{-1}, which are too low for SRB requiring H_2 concentrations of at least 1 - 4 × 10^{-6} mmol l^{-1} to become active. The latter concentration range is, in turn, prohibitively low for methanogenesis, which is characterized by associated H_2 concentrations of 5 - 15 × 10^{-6} mmol l^{-1}. Despite some limitations of the use of H_2 concentrations as an indicator of redox zonation /CHA 96b/, /GIE 99/, it has been shown to perform much better than measurements of redox potential in subsurface sedimentary environments /LOV 88/, /LOV 94/, /CHA 96b/.

A further complication to the model of rather clearly discriminated zones of predomination of single microbial reduction processes as shown in Fig. 7.1 comes about with the possibility that microbes responsible for different reduction processes are simultaneously active in the same parts of subsurface environments. Extensive evidence suggests that methanogenesis can commonly occur in sulphate-rich sediments with active sulphate reduction, which contradicts the generally accepted redox sequence prescribing sulphate depletion in advance of active methanogenesis /DHO 02/.

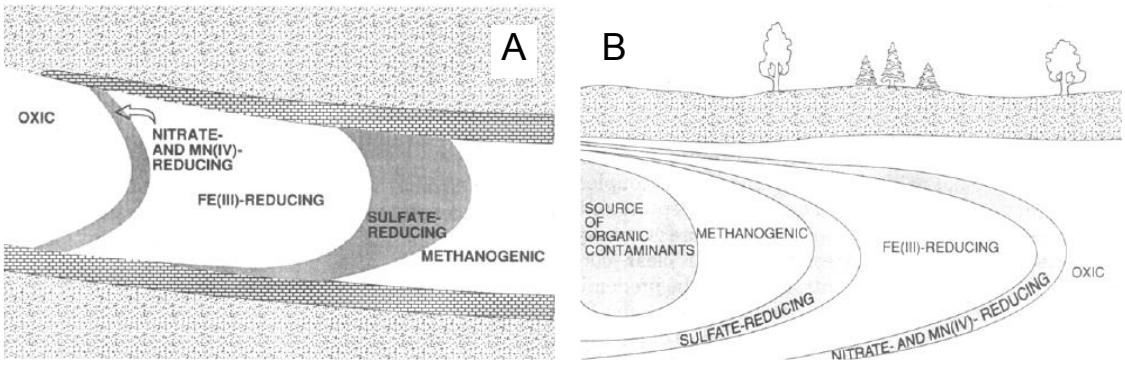

Fig. 7.1 Schematic distribution of microbial reduction processes along the direction of groundwater flow (from left to right) in a pristine (A) or contaminated (B) subsurface environment /LOV 94/

The latter study specifies the following possible reasons for such concomitant occurrence of the competing microbial reduction processes: (i) reliance on different electron donors, (ii) adaptation to lower rates of production of dissolved electron donors, (iii) deviation of the relative in situ free energies for involved reactions from the standard-state free energies, (iv) different susceptibility to viruses. Experimental studies discussed below demonstrate further that some additional reasons can be responsible for violations of the redox sequence in subsurface environments.

A disposal of liquid radioactive waste, which contained salts of acetate and sulphate, in a deep sand–clay formation in Zheleznogorsk, Russia, resulted in methanogenesis that occurred along with – albeit at 3 to ~240 times lower rates than – sulphate reduction /NAZ 04/. In contrast, acetate in a shallow, gas condensate-contaminated sandy aquifer lying above a natural gas field in Colorado, USA, has been found to be predominantly utilized by methanogens with methane accounting for 70 to 100 % of the microbially produced gas /STR 05/. As a result of the contamination, contents of dissolved oxygen, nitrate, and of solid-state Fe(III) were strongly decreased with the latter becoming undetectable, whereas methane concentrations in the groundwater reached values of up to ~1.1 mmol l^{-1} /GIE 99/. In this subsurface setting, methanogens appeared to out-compete acetate-utilizing SRB for acetate despite the presence of sulphate and the significant SRB activity resulting in sulphide concentrations of up to ~0.3 mmol l^{-1} /STR 05/. This rather unexpected finding has been concluded to result from a competition between SRB species for limiting sulphate as terminal electron acceptor with hydrogen-utilizing SRB being more successful competitors than acetate-utilizing SRB.

Similarly, in a shallow sandy aquifer contaminated by crude oil in Minnesota, USA, methanogenesis has been identified as a major process along with the reduction of solid-state Fe(III) in an anoxic zone, which was formed as a result of microbial hydrocarbon oxidation and the related, at least 5,000-fold decrease of groundwater O_2 concentration within about one year after the oil spill /BAE 93/. Acetate produced by fermentative microbes predominately closest to the oil body allowed a concomitant increase of CH_4 and Fe(II) groundwater concentrations by 100 and 25 times, respectively, within the sampled five-year period. Whereas methane concentration of <0.5 µmol l^{-1} was characteristic of the uncontaminated, oxygenated groundwater, it reached the values of up to ~1.6 mmol l^{-1} in the most stable part of the anoxic, contaminated zone about 2 m below the oil body. As high Fe(II) concentrations as of ~1 mmol l^{-1} has been recorded for the same location because of low sulphate concentrations characteristic of the aquifer, which were not sufficient to maintain production of comparable concentrations of sulphide by SRB likewise active in the zone.

Furthermore, the in situ borehole experiment in the Opalinus Clay has revealed that a concomitant activity of methanogens, SRB and fermentative microbes can occur in this clay formation in the excess of electron-donors, which resulted in considerable increases of methane, sulphide, and acetate concentrations of up to 0.7, 1.0, and 13.2 mmol l^{-1}, respectively /WER 11a/. The degradation of the electron-donor source within first two years of the experiment was followed by a consumption of acetate, which was accompanied by an about thirty-fold increase of methane concentration and an about twenty-fold decrease of sulphide concentration. This observation suggests that in this subsurface setting – similarly to the sandy aquifer in Colorado, USA, discussed above – methanogens are able to out-compete acetate-utilizing SRB for acetate despite the significant SRB activity.

Although the near depletion of available organic carbon occurred in the borehole after three years of the experiment, sulphate reduction proceeded at largely unchanged rate for the next two years till the end of the experiment. The source of organic carbon during this stage being not clear, it has been proposed that some insoluble organic species might be responsible for that /WER 11a/. However, this is not necessarily the case, as SRB have been demonstrated to survive and to actively reduce sulphate for at least two months in the absence of a carbon source /SHE 11/. In fact, a number of mesophilic and thermophilic SRB species isolated from deep subsurface environments

utilize H_2 as electron donor with only CO_2 as carbon source as reported, e.g., in /ROZ 01/ and /KAK 06/.

In Late Eocene (~35 million years old) and Late Paleocene (~55 million years old) clay formations in Georgia, USA, methane production has been inferred to be the predominant microbial reduction process at four out of seven sampled locations despite available Fe(III) /SHE 05/. These locations were characterized by organic carbon contents of 0.6 - 2.5 weight %. Yet at another location with organic carbon content of 5.3 weight %, sulphate reduction predominated. Fe(III)-reducing microbes were present at all these locations as has been demonstrated by a stimulation of their activity using a Fe chelator. On the contrary, two remaining locations with comparable amounts of Fe(III), but characterized by at least one order of magnitude lower organic carbon contents of 0.06 - 0.07 weight %, have featured Fe(III) reduction as the predominant microbial process. According to these observations, the availability of electron donors in excess of that of electron acceptors or, alternatively, the decreased accessibility of the latter as compared to that of the former can be a principal factor governing which microbial reduction process predominates in a geological setting.

Upon an increase of temperature from mesophilic (e.g., 37 °C) to thermophilic (e.g., 55 °C) values and at oversupply of electron donors, a shift from acetate-utilizing methanogenesis to that utilizing H_2 and CO_2 can occur in the presence of sulphate with the methanogens outcompeting SRB for H_2 /PEN 04/. This is in contrast to commonly assumed ability of hydrogen-utilizing SRB to rapidly out-compete hydrogen-utilizing methanogens /MUY 08/. To explain this observation, acetate conversion to CO_2 and H_2 by fermentative microbes has been suggested (in a reaction reverse to the reaction (*3*) given in the summary of the specifications of the safety-relevant process “Microbial gas production”).

On the contrary, a stimulation of acetate formation from CO_2 and H_2 by fermentative microbes at thermophilic and hyperthermophilic temperatures above 50 °C has been concluded for the mineral and rock amended sediments, in which methanogenesis occurred in the presence of sulphate /PAR 11/. This finding demonstrates that H_2 formation induced by microbial processes as discussed in the preceding section can suffice for the concomitant activity of fermentative, methanogenic and SRB species competing for H_2 as electron donor. Yet, an accumulation of acetate accompanied by a strong inhibition of methanogenesis and sulphate reduction has been observed in the

latter study at temperatures above 70-80 °C. A possible reason for this observation can be that methanogens and SRB may be not capable of metabolizing acetate in such hot environments, as only Fe(III)-reducing microbes are known to be capable of that so far /LOV 04/.

In the OPHELIE mock-up experiment, discussed in detail for the safety-relevant processes “Microbially influenced corrosion and activity of sulphate-reducing bacteria”, both natural organic matter of the clay component of the backfill and graphite added in the latter to improve thermal conductivity have been suggested as possible sources of organic carbon for the observed microbial activity /DEC 04/. Additionally, Glötzl hydraulic load cells used in the experiment to measure the total pressure have been found to be totally destroyed and to have released about 400 ml of a mixture of oil dissolved in gasoline into the mock-up /VAN 09/. In fact, subsurface anaerobic microbes have since been revealed to be able to utilize graphite as electron donor /GRE 04/. Noteworthy, the graphite tip of an electrode used for a continuous monitoring of electrical conductivity of the borehole solution in the in situ experiment in Opalinus Clay has been almost completely leached within several months /WER 11a/, which may be an indication of the microbial utilization of graphite.

Considering that (i) graphite amounted to 5 % of the backfill, (ii) the formation of the further electron donor H_2 is possible as discussed above, and (iii) gypsum and sulphate were abundant in the backfill /DEC 04/, /RAY 04/, the setting of the OPHELIE experiment cannot be reasonably assumed to have been either an electron-acceptor or an electron-donor limited one. The high activity of SRB, similarly high populations of SRB and methanogens (>1.5 10^5 ml^{-1}) in the hydration circuit maintained in the direct contact with pore water of the backfill, and lacking microbial competition for nutrients /DEC 04/ may then indicate that either (i) an excess of electron donors may have been characteristic of the OPHELIE experiment similarly to the observations discussed in the three preceding paragraphs or (ii) one of the four possible reasons identified in the above-discussed study /DHO 02/ may be responsible for the concomitant occurrence of methanogenesis and sulphate reduction in the OPHELIE mock-up experiment.

In addition to the above discussed sources of electron donors able to make a subsurface setting limited in electron-acceptors, radiolytic production of H_2 from water is a mechanism that can provide a long-lived and very powerful energy supply for hydrogen-utilizing microbes. Primordial radionuclides and their radioactive decay and fission

products have been revealed to be responsible for H_2 concentrations of up to 1,500 mmol l^{-1} in fracture waters collected at depths of up to 3 km below land surface in the Witwatersrand Basin in South Africa /LIN 05/. Concentration of H_2 at a depth of 768 m has been estimated to be at least 0.1 mmol l^{-1} with a substantial amount in excess of this content being consumed by methanogens to produce CH_4 concentration of ~35 mmol l^{-1}.

It can be concluded from the above discussion that quantifying inventories of electron donors and electron acceptors both indigenous to a geological formation chosen to host a DGR and those to be introduced there during the excavation, operation, and closure of a DGR is indispensable for predicting which microbial processes may be active or predominate at which locations within the containment-providing rock zone and at which times after the DGR closure. Such predicting is in turn a prerequisite for carrying out a detailed quantitative analysis of the maximum possible effects of microbial activity in a DGR and can therefore be supposed to become an integrated part of the procedures for assessing long-term performance of a DGR.

8 Summary and research agenda

The primary purpose of the present work was to qualitatively evaluate the relevance of microbial activity for the long-term performance of a DGR and to identify which safety-relevant processes and properties can be potentially influenced by this activity. This work should also provide a basis for the quantitative estimation of the maximum possible effects of microbial processes on the barrier system of a DGR as well as for the consideration of microbial impact on radionuclide redox chemistry and transport in DGR environments in future work. The present analysis identified eight clay properties essential for maintaining safety functions of containment and retardation /DEC 08/, /SKB 11/ of the disposal system – swelling pressure, specific surface area, cation exchange capacity, anion sorption capacity, porosity, permeability, fluid pressure, plasticity – which can potentially be influenced by microbial processes in clay buffer and claystone within a DGR for HLW/SF.

Radioactive waste canisters and over-packs made either of cast metal, carbon steel or stainless steel represent a further component of the engineered barrier system which can be strongly affected by microbial activity in clay buffer or in adjacent host rock. According to the current state of knowledge, iron(III)-reducing, sulphate-reducing, fermentative, methane-producing, and methane-oxidizing microbes can be considered to be present in any clay formation to be utilized either as a source of clay buffer material or as a host rock for a DGR for HLW/SF. The growing body of observations suggests also that each habitat includes a massive number of microbial niches with perhaps only a small proportion of the species being metabolically active at the habitat's conditions, the remainder becoming not extinct – which makes microbes discontinuously different from larger organisms /PAT 09/. Thus, e.g., hyperthermophilic microbial species are available in the currently rather cold environments, such as deep clay formations, and can become active as soon as temperatures in a DGR increase as a result of the placement of HLW/SF.

Moreover, it can further be concluded from the available experimental data that clays contain electron donors and electron acceptors in amounts sufficient for these microbes to remain active – even though perhaps at low metabolic rates – during very long periods of time. Additional sources of electron donors or electron acceptors will inevitably be added to the repository system as a result of DGR excavation, placement of radioactive waste as well as backfilling and sealing of the DGR. In this regard, it is important to bear in mind that the ability of microbes to use sophisticated systems to

access distant electron donors and acceptors will ease a possible limitation of their local availability on activity of microbial population. A schematic summary of possible sources of electron donors and acceptors identified in the present analysis for a DGR in clay as well as of microbial metabolism products of major importance for its long-term performance is given in Fig. 8.1.

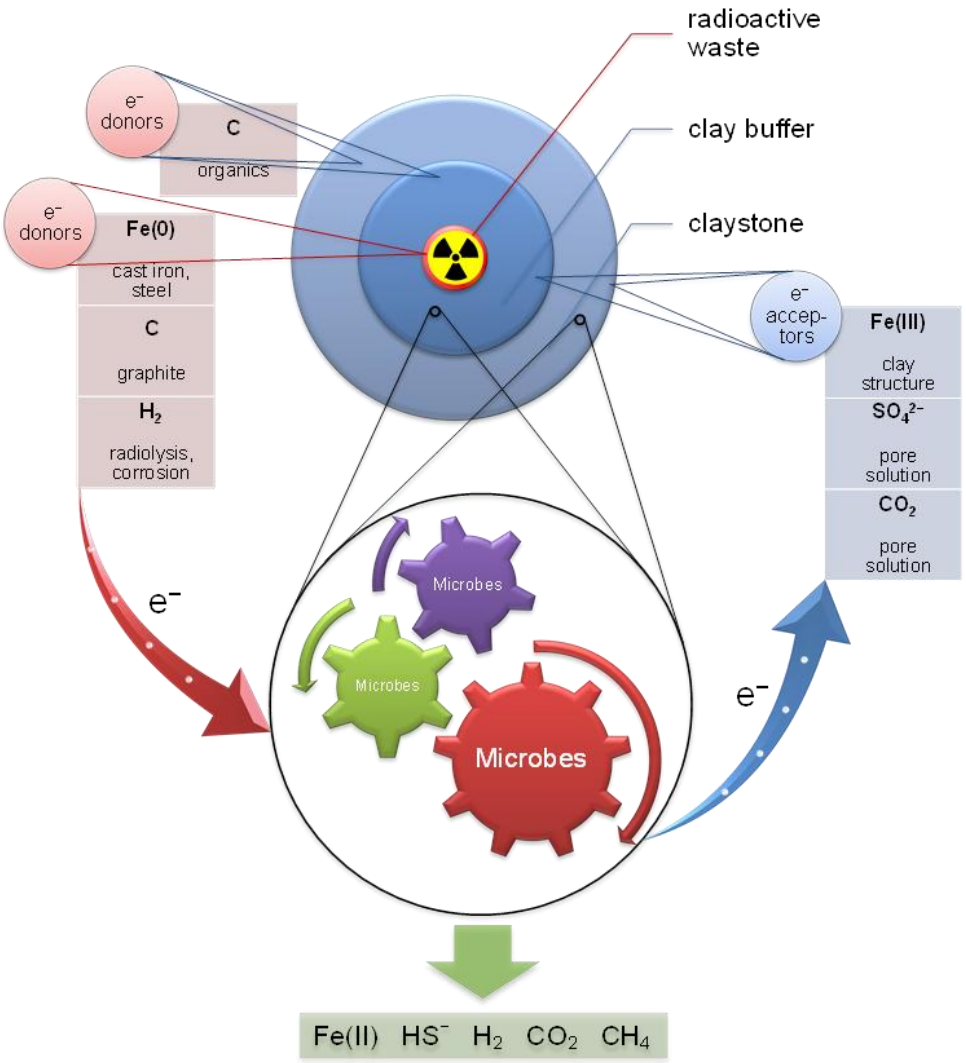

Fig. 8.1 Schematic summary of sources of electron donors and acceptors for microbial activity in a DGR in clay as well as of microbial metabolism products of major importance for its long-term performance

Structural Fe(III) of clay mineral layers represents the primary electron-accepting reactant in redox reactions driven by Fe(III)-reducing and methane-producing microbes as well as by sulphate-reducing microbes in the case of limited supply of sulphate. However, even in the case that the latter microbes predominantly use sulphate as the terminal electron acceptor, the product of their metabolism, hydrogen sulphide, reacts with structural Fe(III) in an abiotic redox reaction, which is facilitated by further, organic products of SRB metabolism. Accordingly, deterioration of clay properties accompanying destabilisation and transformation of clay structure as a result of microbial actions aimed at reducing Fe(III) to Fe(II) directly in the mineral structure or at dissolving Fe(III) and making it available for intracellular redox reactions can be considered as the primary microbial impact on clay.

Microbial reduction of structural Fe(III) has so far been demonstrated to produce up to ~1 - 2 mmol structural Fe(II) per g clay by a solid-state transformation pathway alone and to significantly deteriorate clay's swelling pressure, cation exchange capacity, and specific surface area while improving clay's anion sorption capacity. As a result of the production of ~1.2 mmol structural Fe(II) per g clay – either through direct reduction by microbes or through a reaction with the microbially produced hydrogen sulphide – the clay structure becomes destabilized and a release of Fe(II) accompanied by releases of other structural ions of clay begins. Another clay-dissolution pathway in the presence of microbes proceeds by a direct chelation and solubilisation of structural Fe(III) by microbially produced, low-molecular-weight organic acids, siderophores, and possibly flavins. Microbial production of chelating compounds in the repository environment was recently judged particularly important in the safety analysis of radioactive waste disposal while being one of the least understood processes /PED 05/. Their synergistic effect on clay dissolution, which can be assumed to exist based on observations made for Fe(III) (hydr)oxides, is another critical, yet unresolved research issue (Tab. 8.1).

The clay dissolution proceeding by either reductive or non-reductive pathway results in a release of silica and, in the presence of soluble potassium, in the irreversible conversion of swelling smectites to non-swelling illites. The latter reaction is greatly accelerated (from geological timescales to laboratory ones) due to microbial activity. Precipitation of the released silica and the neo-formed illite in pore space can result in a decrease of porosity and permeability of clay as well as in an increase of fluid pressure that can eventually lead to the temporal loss of clay plasticity and to discharge of the clay-confined fluid. As a further consequence of clay dissolution, clay's swelling pres-

sure, cation exchange capacity, and specific surface area become irreversibly deteriorated.

It is important to realize that clay half-lives in subsurface settings can vary between just under one hundred thousand years up to a few million years in the presence of millimolar concentrations of hydrogen sulphide, which have been observed in rock formations considered as potential DGR hosts at the condition of sufficient electron donor availability. These timescales are comparable with the above-mentioned timeframe of one million years relevant for safety analyses, for which an assessment of the safety of a potential DGR for HLW/SF after its closure should be carried out in accordance with regulatory requirements in many countries. Therefore, an estimation of timescales for microbially-driven reduction and dissolution of clay in deep subsurface remains an important subject for future studies (Tab. 8.1).

In a further microbial process, formation of biofilms, clay dissolution can be accelerated by at least one order of magnitude due to an increase of acidity within a biofilm or biofilm-confined pore spaces by up to four orders of magnitude. Another increase in the dissolution rate can be expected to result from a significant reduction of metabolic expense of secreting electron shuttles or Fe(III)-chelators in a biofilm, where they can be effectively reused. Since the extracellular polymer matrix and the boundary of biofilm allow microbes to control the level of metal ions in order to provide optimal metabolic conditions despite possible presence of toxic substances, biofilm formation can lead to accumulation of some metals at levels exceeding those in contacting aqueous solution by up to several orders of magnitude. This accumulation is, however, selective and unique for each specific subsurface environment, so that the possible net effect of biofilm formation on cation and anion sorption capacities of clay can be assumed to depend on the solute identity.

Furthermore, the formation of biofilms can substantially influence mass transport and hydrodynamics in porous media by strongly reducing its porosity and permeability and making it even impermeable within short periods of time as observed in column experiments or during the defueling operations in the damaged Three Mile Island reactor. Still, the issue of biofilm formation in the DGR environment has not been given proper attention in the scientific literature, which leaves the questions on whether the biofilms can form in clay buffer, claystone or at their interfaces and on how large can be their effect on clay dissolution largely unanswered (Tab. 8.1).

The formation of biofilms also favours – but is not a prerequisite for – the occurrence of the process of microbially influenced corrosion, which is most closely identified with activity of sulphate-reducing bacteria. The ability of SRB to accelerate corrosion of waste canister or over-pack materials has been so far a major concern of microbial research related to final disposal of radioactive waste in geological formations. As a result of SRB activity, rates of anaerobic corrosion of iron-based materials can increase by at least two orders of magnitude as compared to abiotic ones and reach as high values as of 700 μm yr^{-1}, and uniform corrosion can change to pitting corrosion. It cannot currently be concluded from the available experimental data whether, and if so to what extent, the change to pitting corrosion is favoured by which one of the identified four mechanisms of microbially influenced corrosion. This exposes a clear research demand on this topic in order to be able to evaluate the possibility of occurrence of pitting corrosion in a DGR for HLW/SF and to quantify its maximum possible effect (Tab. 8.1).

Both an in situ experiment in the Opalinus Clay and a mock-up experiment simulating the waste disposal architecture pursued in Belgium have demonstrated a potential high impact of SRB on pore water chemistry and on performance of metals at repository conditions in disturbed clay. The recent experimental studies revealed that an increase of clay density up to 2.0 g cm^{-3} by compaction can reduce corrosion rates due to SRB activity by an order of magnitude but not to eliminate that. Furthermore, no elimination of SRB activity can be expected as a result of temperatures of up to 100 °C, as they are still conducive for hyperthermophilic SRB present in clays. Owing to the ability of SRB to reduce structural Fe(III) of clay directly or through production of hydrogen sulphide, SRB activity can negatively influence eight safety-relevant properties of clay. This aspect of SRB activity was strongly underrepresented in previous experimental investigations in the field of geological disposal of radioactive waste and clearly requires a proper consideration in the future research activity (Tab. 8.1).

It appears also that in no case should the potential impact of SRB be underestimated based on a possible argument of comparably low biomass of the microbes in contact with metal surfaces or dissolved metals. The timeframe of their potential activity must be considered concomitantly, as in a space of several years a SRB population confined in a relatively small biofilm encountering sub-micrometre concentrations of dissolved metal in a deep subsurface environment is able to immobilize the metal in precipitates in amounts which would correspond to its economic deposit if extrapolated to geologic

scales. Based on observations made for deep subsurface environments, the timeframe of microbial activity in clay formations to be chosen for radioactive waste disposal can be expected to exceed the above-mentioned timeframe of one million years relevant for safety analyses. Therefore, a quantification of the maximum extent of the identified potential effects of microbial activity in clays should give a proper account of long-term changes in the DGR environment which can be of relevance for a possible activation or facilitation of microbial processes unfavourable for the DGR performance (Tab. 8.1).

Production, conversion or consumption of gases in reactions involved into microbial reduction of Fe(III) or sulphate, organics fermentation, reduction of CO_2, and methane oxidation represent a further important issue for the consideration of the overall microbial impact on the long-term performance of radioactive waste repositories in clays. A build-up of gas overpressure around a DGR for HLW/SF because of a disparity between the gas diffusion and gas production rates can result in a fracturing of the clay rock preceded by loss of clay plasticity and accompanied by gas discharge from the DGR. This build-up can be even amplified in a DGR in clay populated with methanogenic microbes, as increased hydrostatic pressures can strongly favour growth and metabolic activity of methanogens. In this case, a DGR may represent a system characterized by a positive feedback with respect to the increase of hydrostatic pressure. Since an in situ experiment in the Opalinus Clay measured microbial methane concentrations comparable to those in hydrocarbon-contaminated subsurface settings where methanogenesis was a major microbial process, future research effort should be aimed at providing data necessary for a quantification of the potential impact of methanogens on the DGR safety (Tab. 8.1).

A further research topic demanding extensive investigation is related to the very recently revealed ability of microbes to induce significant H_2 formation from common minerals and rocks, which may potentially provide additional sources of gas as well as electron donors and acceptors in the repository environment. Thereby, a total microbial gas production utilizing electron donors indigenous to the clay within the containment-providing rock zone and to backfilling and sealing components of a DGR should be set in relation to the gas production as a result of the corrosion of waste canisters and other iron-based materials, which has been considered so far the major source of gas in a DGR.

Tab. 8.1 Research agenda for future work aimed at estimating effects of microbial activity on long-term performance of radioactive waste repositories in clays

Microbial process	Research issue
reduction of clay	reduction rate
dissolution of clay	dissolution rate production and synergistic effect of organic acids, siderophores, and flavins
biofilm formation	occurrence in a subsurface clay environment effect on microbial clay reduction and dissolution
sulphate reduction	pitting corrosion rates for iron-based materials in contact with clays rates of reduction of structural Fe(III) in clay by microbially-produced hydrogen sulphide
gas production	effect of increased (hydrostatic) pressure on activity of methanogens in clays H_2 formation from minerals in the host rock and in backfilling and sealing components of a DGR
all processes	inventories of electron donors and acceptors in a DGR size of microbial population and its dependence on long-term changes in the DGR environment methodological basis for predicting spatial and time predominance of microbial processes in a DGR

While the occurrence of either of the discussed microbial process in the repository environment – even though perhaps at only low metabolic rate – appears to be backed by the available experimental data, the question of their interplay at repository conditions should be considered still open. Contrary to the earlier, simplistic conception of the dominance order of microbial processes, which was based solely on energetic considerations, the present view recognises that their occurrence critically depends on local heterogeneity of microbial habitats with respect to, most importantly, the availability of electron donors and acceptors. Quantifying inventories of electron donors and electron acceptors both indigenous to a geological formation chosen to host a DGR and those to be introduced there during the excavation, operation, and closure of a DGR is indispensable for predicting which microbial processes may be active or predominate at which locations within the containment-providing rock zone and at which times after the DGR closure. Future studies should develop a methodological basis and identify parameters and data required for implementation of such an approach in safety analyses of deep geological repositories (Tab. 8.1).

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