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Microbial processes in a clay repository
Some examples from a recent review of the literature

Cologne, 4 – 5 November 2013
Credit and Acknowledgments

- This presentation is based on a compilation of many results from the literature:


- Many thanks to the colleagues and authors of these different studies from which the examples and illustrations used in this presentation have been borrowed (see /citations/ in the slides and the references list at the end of this presentation)
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● Extremophiles and autotrophic micro-organisms
● Metabolism and conditions required for microbial activity
● Effects of microbial activity for a repository in clay:
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  – Possible but uncontrollable beneficial effects
● How to restrict microbial activity by design?
  – Safety requirements for a deep repository
● Conclusions
Background and scope

- Micro-organisms are very efficient biochemical entities and participate to many important geochemical processes.
- Micro-organisms are shaping the Earth landscape since more than 3.5 billion years (3.5 Ga) and have adapted to very diverse and extreme conditions.
- Bacteria and archea are able to survive (inactive) and to strive (active) under extreme conditions:
  - temperature, pressure, radiation, high salinity, pH, …
- Deep subsurface environments host microbes to a depth of ~ 4 km (gold mines in South Africa, deep sediments, …)
- **Question**: what are the effects of microbial activity for the safety of a deep geologic repository (DGR) ?
Extremophile micro-organisms: high temperature

- **Yellowstone**: thick microbial red coloured mats developing in hot springs and geysers (thermophiles, hyperthermophiles)

Hyperthermophiles

T: 80 – 120 °C

http://exoplanet.as.arizona.edu/~lclose/teaching/a202/lect10.html
MICROBIAL METABOLISM
Microbial metabolism and energy

- Micro-organism’s metabolism transforms matter and energy; they are subject to the laws of thermodynamics

- The free-energy change (\( \Delta G = \Delta H - T\Delta S \)) of a chemical reaction indicates whether, or not, this reaction can spontaneously occur (\( \Delta G < 0 \))

- Enzymes (catalysers) speed up metabolic reactions by lowering energy barriers

- Cellular respiration is a major catabolism pathway releasing energy for the cell activity

→ The chemical effects of microbial processes can be modelled with geochemical codes based on thermodynamics
Cellular respiration provides energy to the cell

- Cellular respiration is a major catabolism pathway releasing energy for the cell activity.
- The best known cell respiration is oxidation of glucose by oxygen to produce CO₂ and H₂O
  
  \[ C_6H_{12}O_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} \]

- Other catabolic pathways yield energy by oxidizing different reductants without the use of oxygen.
- Anaerobic respiration and fermentation enable cells to produce energy without the use of oxygen.
Cell respiration = oxidation / reduction reactions

Anaerobic chemotrophic micro-organisms use other reducing and oxidizing agents for their respiration in a complex interplay of reactions, e.g.:

\[
\begin{align*}
\text{CH}_2\text{O}, \text{H}_2, \text{NH}_3, \text{Fe}^{2+}, \text{HS}^- & \quad \text{NO}_3^-, \text{Fe}^{3+}, \text{SO}_4^{2-}, \text{CO}_2 \\
\text{CO}_2, \text{H}_2\text{O}, \text{NO}_3^-, \text{Fe}^{3+}, \text{SO}_4^{2-} & \quad \text{NH}_3, \text{Fe}^{2+}, \text{HS}^-, \text{CH}_4
\end{align*}
\]

+ release of energy ($\Delta G < 0$) to sustain microbial activity
Redox ladder for respiration of chemo-autotrophic micro-organisms (bacteria & archaea)

<table>
<thead>
<tr>
<th>Eh (mV)</th>
<th>Oxidizing</th>
<th>Aerobic</th>
<th>Aerobic respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 700</td>
<td>O₂ / H₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 600</td>
<td>NO₃⁻ / N₂</td>
<td>Anaerobic</td>
<td>Nitrate reduction</td>
</tr>
<tr>
<td>+ 500</td>
<td>MnO₂ / Mn²⁺</td>
<td></td>
<td>Manganese (IV) reduction</td>
</tr>
<tr>
<td>+ 0</td>
<td>Fe₂O₃ / Fe²⁺</td>
<td></td>
<td>Iron (III) reduction</td>
</tr>
<tr>
<td>- 200</td>
<td>SO₄²⁻ / HS⁻</td>
<td></td>
<td>Sulfate reduction</td>
</tr>
<tr>
<td>- 250</td>
<td>CO₂ / CH₃COOH</td>
<td></td>
<td>Acetogenesis</td>
</tr>
<tr>
<td>- 300</td>
<td>CH₃COOH / CH₄ + CO₂</td>
<td></td>
<td>Fermentation</td>
</tr>
<tr>
<td>- 350</td>
<td>CO₂ / CH₄</td>
<td></td>
<td>Methanogenesis</td>
</tr>
<tr>
<td>- 700</td>
<td>H⁺ / H₂</td>
<td>Reducing</td>
<td>Water proton reduction</td>
</tr>
</tbody>
</table>

(anaerobic corrosion of metals)
Mains conditions required for microbial growth

- Autotrophs are able to synthesize their organic carbon from inorganic carbon (CO$_2$, HCO$_3^-$, CO$_3^{2-}$)
  - chemo-autotrophs, or litho-autotrophs, do not need light as photo-autotrophs using photosynthesis

- Heterotrophs recycle organic carbon produced by autotrophs

- In the deep subsurface environment, chemo-autotrophs (litho-autotrophs) require:
  - Space to grow
  - Water: indispensable solvent for biochemical reactions (no active life is possible without water)
  - Electron donors and acceptors (to free-up energy: $\Delta G < 0$)
  - C-H-O-N atoms to build glucides, lipids, peptides, proteins, enzymes, and their cell outer membrane
  - Micro-nutrients:
    - phosphorus → ATP to store their energy
    - traces of metals (Mg, Fe, Co, Mo, Se, …) needed for enzymatic catalysis
  - Acceptable conditions of T, pH and radiation
Add **space and water** to clay and dormant microbes will become active

- If sufficient space and water are available and pH < 12, microbes are expected to be active and to develop in a deep geologic repository.

- How will microbial activity affect:
  - the geochemical conditions prevailing in the near field and EDZ?
  - the performances of the engineered barriers (metallic overpacks, bentonite, buffer materials, ...)?
  - the solubility, the sorption and the mobility of radionuclides?
  - the fate and transport of gases (H₂, CH₄, CO₂, ...)?

- What are the consequences of a microbial perturbation for the safety functions (containment, confinement) of a DGR?

- How to address microbial processes?
MICROBIAL INFLUENCED CORROSION (MIC)
Microbially Influenced Corrosion (MIC) by hydrogen sulfide (H$_2$S) attack

Sulfate-reducing bacteria (SRB) produce H$_2$S and are responsible for MIC

4 H$_2$ + SO$_4^{2-}$ → S$^{2-}$ + 4 H$_2$O

- MIC reaction for cast iron, carbon steel, or stainless steel:
  Fe + H$_2$S → FeS + H$_2$ (sulfide stress corrosion cracking, SCC)
- MIC does not require a direct contact between sulfate-reducing bacteria (SRB) and the metal surface
- MIC is limited by diffusion of sulfide in compacted bentonite (at dry density > 2 g cm$^{-3}$) 
  (Pedersen, 2010)
Microbially Influenced Corrosion (MIC) by hydrogen sulfide (H_2S) attack

Sulfate-reducing bacteria (SRB) responsible for MIC:

- can be indigenous to clay materials,
- survive and remain active in compacted bentonite up to:
  /Chi Fru et al., 2008; Masurat et al., 2010b/
  - clay density of 2.0 g cm\(^{-3}\),
  - water activity of 0.55 – 0.75 (desiccation),
  - temperature of 120 °C (for at least 15 hours),
- grow at salt concentrations of up to 350 g L\(^{-1}\) /Oren, 2010/,
- actively reduce sulfate in deep-sea sediments with optima at 80 to 95 °C and, possibly, 103 to 106 °C /Jørgensen et al., 1992; Weber et al., 2002/,
- grow by reducing structural Fe(III) of clay in the absence of sulfate /Li et al., 2004/.
MICROBIAL EFFECTS ON CLAY TRANSFORMATIONS
Microbial clay reduction of structural Fe(III)

- In smectite (2:1 layer or TOT phyllosilicate), octahedral gibbsite layers contain structural Fe(III) substituted to Al(III)

- Fe(III) can be reduced into Fe(II) by various bacteria species:
  - Ferri-reducers
  - Sulfate-reducers
  - Methanogens

\[
\begin{align*}
\text{Fe}^{3+} & \xrightarrow{+ \text{dissolved K}^+} \text{Fe}^{2+} \\
\text{TOT} & \rightarrow \text{Illite (non-swelling)}
\end{align*}
\]

\[
\begin{align*}
\text{TOT structure destabilisation} \\
\text{more negative surface charges} \\
\rightarrow \text{collapse of interlayers}
\end{align*}
\]
Microbial reduction of Fe(III) in clays

One of the three currently identified mechanisms: transfer of electrons to structural Fe(III) through a direct contact

Jaisi et al., 2007
Microbial reduction of Fe(III) in clays

One of the three currently identified mechanisms: transfer of electrons to structural Fe(III) through a direct contact or up-to 50 µm long bacterial nanowires

Jaisi et al., 2007  Gorby et al., 2006
Microbial reduction of Fe(III) in clays

One of the three currently identified mechanisms: transfer of electrons to structural Fe(III) through a direct contact or up-to 50 µm long bacterial nanowires

Jaisi et al., 2007
Gorby et al., 2006
Sherar et al., 2011
Microbial reduction of Fe(III) in clays

One of the three currently identified mechanisms: transfer of electrons to structural Fe(III) through a direct contact or up-to 50 µm long bacterial nanowires

Fe(III)-reducing bacteria can be:
- indigenous to clays,
- characterized by optimum growth temperatures of up to 121 °C /Kashefi et al., 2004/,
- responsible for high groundwater concentrations of Fe(II) /Lovley et al., 2004/, and,
- are responsible for the formation of the world’s largest, ~ 40 – 80 million year old deposit of high-brightness kaolin. Its slurries must be routinely treated with a biocide to reduce microbe reproduction below the industry tolerance level /Hurst et al., 1997/.
Microbial clay reduction of structural Fe(III)

Smectite transformed into illite after 2 months with Fe(III)-reducing bacteria. TEM micrographs and XRD pattern indicate a decrease of the interlayer space /Dong et al., 2003/
Geobacter sulfurreducens

Electrically conductive pili enable Geobacter species to perform extracellular electron transfer reactions and to transport electrons to remote iron particles or to other microbes.

Electrically conductive pili ~ nanowires
(www.geobacter.org; Lovley, D.R.)
Pili can conduct electrons over very long distances (cm) (thousands of times the size of the bacterium)
Examples of microbial reducing perturbations induced by SRB fuelled by unexpected sources of organic carbon observed at Mont Terri (Switzerland) and Mol (Belgium)

IN SITU AND LARGE SCALE EXPERIMENTS DISTURBED BY MICROBIAL ACTIVITY
Porewater chemistry (PC) experiment at Mont Terri

Wersin et al. (2011)
Porewater chemistry (PC) experiment

"Due to unexpected trends in pH and other water parameters, microbial analyses were conducted after 9 months, which, for the first time at the Mont Terri URL, confirmed that microbial processes were occurring in a borehole. This led to a change in focus of the analytical program."

Sulfate reduction by continuous electron-donor supply by glycerol from gel-filling of pH and Eh electrodes

Wersin et al. (2011)
Accumulation of sulfate and sulfide behind the concrete lining of the connection gallery (Hades, Mol URL)

Strong H₂S smell after borehole drilling

SRB detected + 1 mM HS⁻

Sulfide in cultured SRB: 1152 mg/L = 36 mM!

pH ~ 5.5 behind concrete in place of expected pH ~ 12.5

For what reason?

H$_2$S produced by SRB fuelled by organic carbon of wood pieces placed behind the concrete lining
HOW TO CONTROL AND TO LIMIT MICROBIAL ACTIVITY?

Space and water restrictions + high pH
How to limit / restrict microbial activity?

Conditions unfavourable for microbial growth:

- **Lack of space:**
  - Because transport of electron donors and acceptors and other nutrients (P, Se, …) is limited by diffusion in compact clay materials:
    - Microbial activity stops during clay formation diagenesis when the degree of compaction becomes too high (Lerouge et al., 2011)
    - Microbes cannot easily develop in highly compacted pure bentonite (100 % bentonite) at elevated dry density ($\rho_{\text{dry}} > 2 \, \text{g/cm}^3$)

- **Lack of water:**
  - Microbial activity stops if water activity is < 0.6 because of osmotic effects in the cell induced by desiccation

- **High pH:**
  - Microbial activity stops at pH > 12 – 12.5 (cementitious materials)
“Space Restriction”: An essential safety requirement to minimize microbial activity

FANC safety requirement for geological disposal:

“The presence of voids inside a repository and their dimensions (pore size) must be sufficiently small:

– to avoid to jeopardize the mechanical stability of the disposal system;
– to limit preferential paths for radionuclides and water flow, and;
– to prevent microbial activity.”
Conclusions

- Beside man-introduced bacteria, dormant indigenous microbes encapsulated since tenths of million years in compact deep geological formations may have been detected, even if large uncertainties sometimes remain.

- If sufficient **space and water** are available in a deep repository, dormant microbes can become active and can deteriorate engineered barriers.

- Limitation of voids and their dimensions (pore size) in a repository is an essential safety requirement to prevent microbial development and its potential detrimental effects.

- Microbes can spoil long-term geochemical experiments and microbial processes cannot be ignored for metal corrosion.
Thank you for your attention

Questions?

Tetrad of *Deinococcus radiodurans*  
(Image: Wikipedia Commons, Public Domain)
~5-μm-wide cluster of square, <0.1 μm thick, halophilic microbes /Boetius et al., 2009/.

http://www.grs.de/publication/GRS-291
References (1/3)

References (2/3)


- /Lydmark S. and Persson J., 2010/: Production of gas and sulphide by bacteria in Boom Clay. NIROND-TR 2010-16E.


EUROSAFE
References (3/3)


- NWMO, 2005/: Choosing a way forward. The future management of Canada’s used nuclear fuel (Final Study). Nuclear Waste Management Organization (NWMO), 2005.


REFERENCES
RESERVE
Extreme forms of life: high T and P (2250 m)

- Hydrothermal vents under ocean floor, near magmatic basaltic ridges (black smokers with metallic sulfides emanations)

Steep T gradient: bacteria, worms, and a complex food web (strain 121)

Images: courtesy of NOAA and University of Victoria
Halomonas titanicae sp. nov.: a halophilic iron oxidising bacteria isolated from the RMS Titanic

Rusticles: (rust + icicle) microbial colonies active on the RMS Titanic hull in anoxic water (-3900 m) and “eating” its metallic parts (DNA identified in 2010)

Sànchez-Porro et al. (2010) and National Geographic Society
CONDITIONS FOR MICROBIAL ACTIVITY
Other conditions required for microbial growth

- Acceptable **pH range**: 0 → 12
  - Cell membrane proton pumps work is too energetic at pH > 12
  - Cell membrane hydrolysis under very acidic and hyper-alkaline conditions

- Acceptable **T range**: 0 → 120 °C
  - Hyper-thermophiles strive at 80 – 120 °C
  - Proteins and DNA denature at T > 150 °C

- Acceptable **radiation dose range**:
  - *Deinococcus radiodurans* and other microbial strains can resist to very high dose (up to 15 000 Gy) because of their ability to efficiently repair their very compact DNA
MICROBIAL EFFECTS ON A DEEP GEOLOGIC REPOSITORY
<table>
<thead>
<tr>
<th>Component</th>
<th>Safety function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste form (glass matrix, fuel matrix,</td>
<td>Confinement, containment of radionuclides (RN) in waste matrix</td>
</tr>
<tr>
<td>immobilisation matrix)</td>
<td>Attenuation of RN release: low dissolution rate</td>
</tr>
<tr>
<td>Metallic canisters and overpacks</td>
<td>Confinement, prevents water inflow and release of RN</td>
</tr>
<tr>
<td></td>
<td>Attenuation of RN release: <strong>corrosion</strong> products act as reducing agent (→ low RN solubility) and sorbent for RN</td>
</tr>
<tr>
<td>Bentonite buffer and sealing materials</td>
<td>Confinement: long resaturation time</td>
</tr>
<tr>
<td></td>
<td><strong>Plasticity: self-sealing, porosity clogging</strong></td>
</tr>
<tr>
<td></td>
<td>Delay and attenuation of RN release: slow diffusion + retardation (sorption)</td>
</tr>
<tr>
<td>Cementitious buffer</td>
<td>Confinement</td>
</tr>
<tr>
<td></td>
<td>High pH: low solubility and high sorption of RN; limit microbial activity</td>
</tr>
<tr>
<td></td>
<td>Delay and attenuation of RN release</td>
</tr>
<tr>
<td>Geological barriers (host rock + geosphere)</td>
<td>Geochemical and geomechanical stability, isolation,</td>
</tr>
<tr>
<td></td>
<td>Confinement: delay and attenuation of RN release slow</td>
</tr>
<tr>
<td></td>
<td>diffusion + retardation (sorption)</td>
</tr>
</tbody>
</table>
Microbial clay reduction of structural Fe(III)

- Depends on the mechanism of bacterial interaction with clay minerals (a factor of 2–4 /Lovley et al., 1998/ /Jaisi et al., 2008/):

NAu-2 \( (Na_{0.72}(Si_{7.55}Al_{0.45})(Fe(III)_{3.83}Mg_{0.05})O_{20}(OH)_4) \) with Fe(III)-reducing bacteria and electron shuttles (AQDS: 2,6-anthraquinone disulfonate) /Jaisi et al., 2008/
Microbial clay reduction: sulfate-reducing bacteria

- Nontronite NAu-2 + sulfate-reducing bacteria /Liu et al., 2012/:
Microbial clay reduction: methanogens

Nontronite NAu-2 + methanogens /Zhang et al., 2012/:
Smectite‒to‒illite transformation

Microbial Fe(III) reduction results – in the presence of soluble K⁺ – in the irreversible transformation of smectite to illite (either by solid-state or dissolution-precipitation pathway). This reaction requires:

– without microbial activity:
  • 0.5 – 300 million years at 50 – 180 °C /Pollastro, 1993/, or
  • 4 – 5 months at 300 – 350 °C and 100 MPa /Kim et al., 2004/,
– or with activity of Fe(III)-reducing bacteria:
  • a timescale of early diagenesis at 26 to 69 °C in mudstones /Vorhies et al., 2009/
  • 14 days at 25 °C and 0.1 MPa in the lab /Kim et al., 2004/ or

Kim et al. (2004)
Microbial clay reduction of structural Fe(III)

SWa-1 after 2 months with Fe(III)-reducing bacteria

/Dong et al., 2003/
Microbial clay reductive dissolution

Microbial Fe(III) reduction is considered to proceed by a solid-state transformation up to a reduction of ~1.2 mmol Fe(III) per g clay. At higher reduction levels, smectite structure destabilizes and a release of structural Fe(II) becomes possible /Jaisi et al., 2008/.

~ 20 million years old mudstones from Madrid Basin /Sanz-Montero et al., 2009/
OPHELIE mock-up experiment at Mol (Belgium)

Thermo-Hydro-Mechanical (THM) experiment
Fo-Ca clay bentonite (beidellite / kaolinite)
heated up to 120 °C during 5 years

Verstricht et al., 2004
OPHELIE mock-up experiment: corrosion of the tip of heating tube (sand + FeS$_2$ + 0.5 M sulfide)

SRB activity fuelled by organic carbon (fuel + oil) from defective Glötzl cells → 0.5 mM HS$^-$ → 0.5 mM S$_2$O$_3^{2-}$ ➔ Microbial Induced Corrosion (MIC)

SRB: 1.5 E5 cell/mL sulfide (SEM)

Kursten et al. (2004)
CONCLUSIONS
Summary

- Activity of Fe(III)- and sulfate-reducing bacteria as well as of methanogens can lead to clay reduction and alteration.

- Bacteria could be of indigenous origin or introduced by man activities (uncontrollable contamination).

- Sources of electron donors and acceptors necessary for microbial activity will inevitably be added to the repository system as a result of repository excavation as well as placement of radioactive waste, backfill and sealing materials.

- The maximum possible effect of microbial activity on long-term performance of engineered barriers needs to be assessed.
Conclusions (1/3)

- Since ~ mid-1980 many studies have been conducted on the potential effects of microbial activity on radioactive waste disposal in crystalline rocks and clay sedimentary formations.

- Since the breakthrough of the Polymerase Chain Reaction (PCR) in 1983 to amplify DNA, the field of subsurface microbiology has been revolutionised by molecular genetics and abundant information has been gathered over 30 years.

- The big picture of the microbial activity impact for radioactive waste disposal progressively developed over 30 years of research is confirmed by recent works and the bottom approach defined since the end 80’s to avoid microbial activity for a safe repository design remains valid.
Conclusions (3/3)

- Limitation of voids and their dimensions (pore size) in a repository is an essential safety requirement to prevent microbial development and its potential detrimental effects:
  - If sufficient **space and water** are available in a deep repository, microbes are expected to be at “rendezvous” and to deteriorate engineered barriers
  - To avoid microbial activity, a safe and robust repository design requires not to leave voids and to decrease as much as possible the pore size of buffer materials around waste canisters / overpacks (vitrified HLW and spent fuel)
Acknowledgments

- This presentation is the fruit of a compilation of many research studies.

- Many thanks to the different authors and colleagues from which the examples and illustrations used in this presentation have been borrowed (see citations in the slides and the references list hereafter).

- Special thanks to colleagues of the Mont Terri project (Switzerland) and of the HADES underground research laboratory (Euridice EIG, Mol, Belgium) for the many experiences shared.
POROSITY AND BIOFILMS
Most of the pore volume consists of small pores (R < 10 nm)

Compaction decreases the pore volume of large pores

Microbes (micrometre-sized) remain viable in compacted clay but their activity is strongly decreased or inhibited at high degree of compaction

Hicher et al., 2000

/Stroes-Gascoyne et al., 2007/
Biofilms and permeability

Biofilm of indigenous sulfate-reducing bacteria (SRB) – encrusted with neo-formed clay minerals – on crushed Äspö granodiorite in contact with synthetic Äspö groundwater makes packed columns poorly permeable within five days /Tuck et al., 2006/. 
“... in a space of several years sulfate-reducing bacteria (SRB) encountering sub-μM levels of dissolved Zn deposited a ZnS rich biofilm that if continued over geologic time would produce an economic deposit.”

MacLean et al. (2007)
BENEFICIAL EFFECTS OF MICROBIAL ACTIVITY ARE POSSIBLE BUT UNCONTROLLABLE
Possible **beneficial but uncontrollable effects of microbial activity**

Beside harmful impact, microbes could also have positive but uncontrollable effects:

- **Decrease of hydrogen gas pressure** in a clay repository:
  (Mont Terri Hydrogen Transfer (HT) experiment)
  \[ 4 \text{H}_2 + \text{SO}_4^{2-} \rightarrow \text{S}^{2-} + 4 \text{H}_2\text{O} \] (sulfate reduction: observed)
  \[ 4 \text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O} \] (methanogenesis: not observed)

- **Reduction of nitrate** from a plume of bituminised MLW:
  (Mont Terri Bitumen Nitrate (BN) experiment)
  \[ 5 \text{H}_2 + 2 \text{NO}_3^- + 2 \text{H}^+ \rightarrow \text{N}_2 + 6 \text{H}_2\text{O} \]
  (preservation of reducing conditions in the clay formation to retard redox-sensitive radionuclides: $^{79}\text{Se}$, $^{99}\text{Tc}$, $^{23x}\text{U}$, $^{237}\text{Np}$, $^{239}\text{Pu}$, …)
Beneficial effects of microbial processes are uncontrollable and safety cannot be based on them

- Unfortunately, favourable microbial processes are uncontrollable and unpredictable in a deep repository:
  - It is impossible to guarantee that microbes will be viable and active long enough when they will be needed to ensure the long-term safety of a special section (cell, gallery) of a deep repository
  - Microbial mutations and adaptation on the long term cannot be predicted

- So, it would be a “nice to have” additional feature, but long-term safety cannot be based on unreliable processes
Impact of microbial activity for a clay repository?

- Microbial activity (MA) has **many possible detrimental effects** on the safety functions of a deep repository:
  - Microbially induced corrosion (MIC) of metallic overpacks
  - Enhanced transport of radionuclides (complexation, dissolution)
  - Degradation of clay buffer materials, …

- **Beneficial effects of MA are possible but uncontrollable:**
  - Hydrogen and methane gas pressure decrease
  - Restoration and preservation of reducing conditions, …

⇒ How to tackle microbial issues in the **design** of a repository?

⇒ Need of **design requirements** to guarantee the long-term safety of a deep repository