



## **DELIVERABLE D2.17**

### **Sulphide production**

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## **Publishable Summary**

This report summarizes the outcome of the Task 2.1 dealing with the availability of sulphide in the bedrock around the repository. Sulphide is an important constituent affecting metal corrosion and, consequently, processes controlling the access of sulphide into the repository near field must be understood and quantified. Sulphide production is dealt here in a broad sense including the natural presence of sulphide in bedrock groundwater, release from sulphide minerals and transport to the repository. The final aim of this study is to quantify the contribution of microbial sulphate reduction to sulphide.

Lack of sulphur sources can hardly be considered as a limiting factor, even though sulphur-bearing minerals are not always common in all rock types, as for example in granitic rocks. Aqueous sulphate is not solubility-limited, it is a common constituent of near-surface waters and provides the source for microbial sulphide formation, if coming in contact with deep anoxic zone.

Redox conditions of the groundwater-bedrock system are considered as a qualitative measure of the sulphide production capacity of the system. Very low redox values indicate the abundance of electron donors, whereas high redox potentials indicate the presence of oxygen or other strong electron acceptors suppressing the sulphate reduction to sulphide.

Solubility limitation provides the general abiotic constraint on the concentration of sulphide in the groundwater-bedrock system. Relatively high sulphide concentrations occasionally observed in deep groundwaters are evidently due to active microbial sulphate reduction fronts, but transient in space and time.

Presence of electron donors is one of the most important controlling factors for microbial sulphide formation. Dissolved molecular hydrogen is the most effective electron donor, whereas methane is the most common one. Currently, more attention has been paid to the role of organic C-2 compounds, especially acetate.

Nutrient limitation is not considered as an important limiting factor, because the bedrock environment provides an adequate source. In deep biosphere, recycling of essential elements and syntrophic interactions play key roles in sustaining microbial life.

Transport limitations, based on advection and diffusion, are easily quantified ways to estimate long-term sulphide production rates. Ideal conditions in terms of all other factors required by the microbial sulphide production would evidently lead to limited supply by the limited transport rate.

Energetic calculations demonstrate that life deep in the bedrock has a plausible energy source supplied by the surrounding chemical energy. The life-sustaining energy flux, i.e. power, remains however low, and cannot be multiplied by many orders of magnitude in a sustainable way.

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# 1 Introduction

Sulphide has been identified as the main component able to react with metallic copper in anoxic conditions, a conclusion that can be drawn purely from chemical and thermodynamic considerations. However, geochemical processes of sulphur in the bedrock are known to be strongly linked to microbial processes. Microbes catalyse many processes that would be slow in abiotic conditions, because their enzymatic systems are evolved to transfer electrons between molecules that would be otherwise unreactive. In other words, microbes are able to sustain life and grow by releasing chemical energy stored in compounds that are not in thermodynamic equilibrium with their surroundings.

Chemical reactions in general, and reduction-oxidation reactions in particular, deal with electrons. Metal corrosion research is a typical discipline utilizing electrochemical conceptualisations and study methods. Figure 1 gives a schematic geomicrobiological overview of the disposal system consisting of waste canister, buffer and the surrounding biogeosphere. MIND Task 2.1 aims at identifying and quantifying the factors controlling microbial production of sulphide in the geosphere, i.e. the rock mass outside the bentonite buffer primarily protecting the canister. Geochemical and mass transfer constraints to be estimated include 1) availability of sulphur sources; 2) electron donors facilitating sulphate reduction to sulphide; 3) essential and possibly growth-limiting nutrients of the sulphur cycle microbes (S-reducers, S-oxidizers); 4) availability and reactivity of sulphide minerals as sulphide source or sink. The role and properties of weak electron acceptors (e.g. ferric iron) needs also be analysed in detail, because some oxidative sulphide disintegration may take place in the absence of oxygen and other strong electron acceptors. The balance between electron acceptors and electron donors in the system can be described by means of the concept reduction-oxidation potential (redox).

Another aspects to be considered are the spatial dimensions and fluxes: molecular diffusion may play a role in the near field and large-scale hydrological cycle may affect the geochemical conditions of the repository environment in the long-time perspective. Thermodynamics set the ultimate constraint to all chemical processes including the biochemical processes. Life without an energy source is not possible. We have also focussed on understanding of the energetic limits of the deep life, and major progress was achieved in setting time and energy into a single frame.

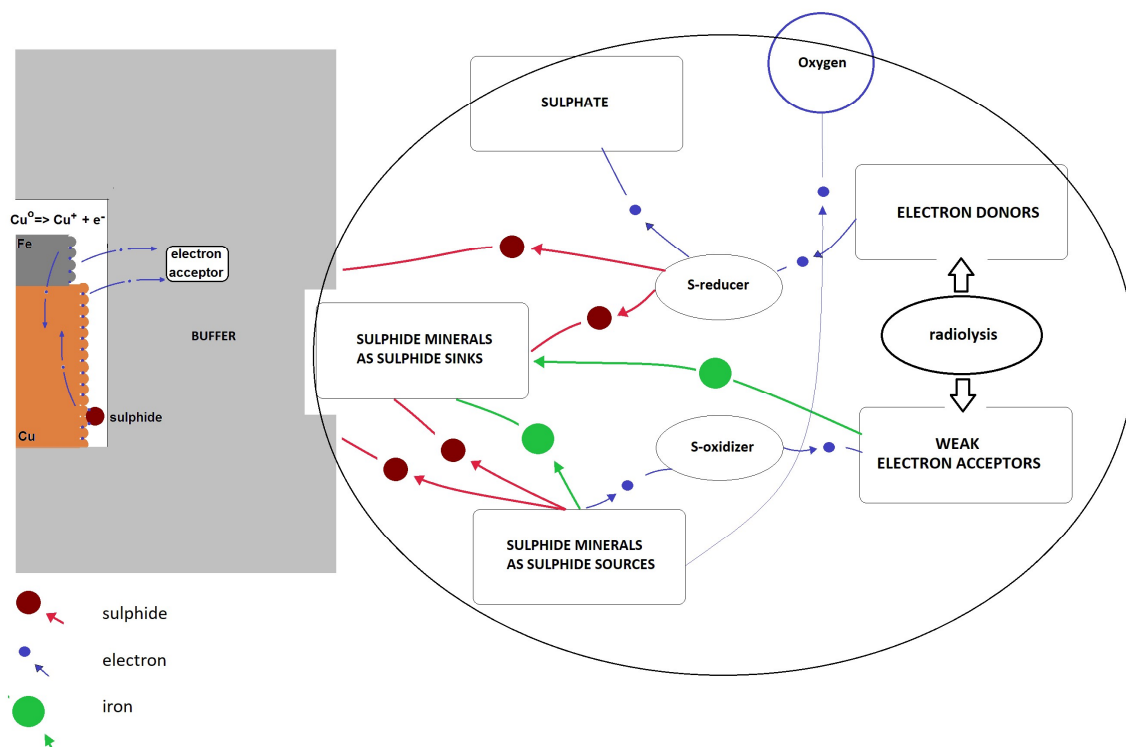


Figure 1. Biogeochemistry of sulphur in the geosphere around the repository

## 2 Redox control

Thermodynamics dictate the energy availability of metabolic reactions. According to thermodynamic ladder, aerobes, nitrate, manganese and iron reducers use their energetic advance to preclude sulphate reduction, and sulphate reducers exclude methanogens. However, the distribution of microbial life in the subsurface is controlled rather by ecologic and physiologic factors than by thermodynamics. Microbes regulate redox conditions of their environment as they oxidize electron donors and reduce acceptors (Bethke *et al.* 2011). That is, microbes obey the thermodynamic laws, but other factors such as hydraulic conductivity and connectivity as well as residence time of water and therefore the openness and dynamism of the groundwater system governs the availability of electron donors and acceptors. In practice this is seen as variable hydrogeochemical conditions and microbial communities in different fracture systems even at one site, and differences in redox depth profiles between different sites. The thickness of the more dynamic and oxic groundwater horizon can in some cases reach depths of several hundreds of metres while in other sites anoxic waters prevail at very shallow levels.

During the construction phase of a repository, oxygen will be present, but oxygen levels will begin to decrease after the closure, providing more favourable niches for sulphate reducers.

## 3 Sulphur sources and sinks

### 3.1 Dissolution of sulphide and sulphate minerals

Dissolved sulphide is a common constituent in anoxic groundwater systems, but due to the complicated sampling and sample preservation the actual concentrations are not often reported. In general, sulphide concentrations in deep anoxic waters seem to be controlled by the solubility of iron mono-sulphide minerals. However, there are observations apparently indicating supersaturation (Figure 2).

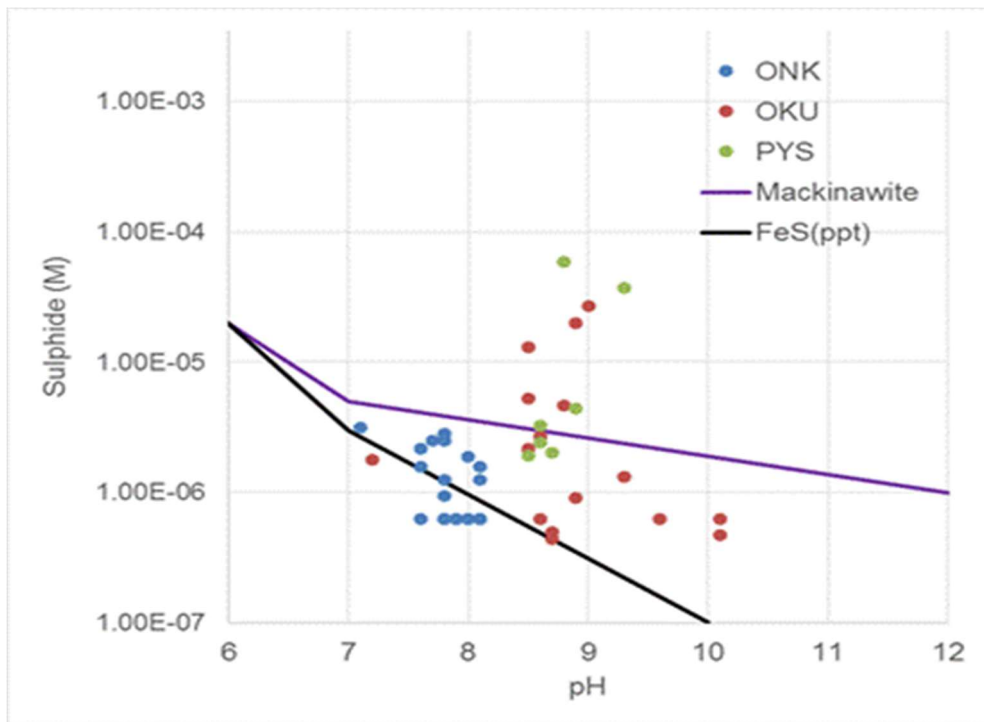


Figure 2. Sulphide solubility as a function of pH assuming that sulphide concentration  $\approx$  iron concentration.

### 3.2 Inorganic sulphur oxidation

**Ferric iron** is an effective electron acceptor possibly oxidizing sulphide minerals. However, compared to oxygen, Fe(III) is a weak oxidizer leading to the formation of metastable sulphur compounds at oxidation numbers between the stable +VI (sulphate), -II (sulphide) and -I (pyrite). Elemental sulphur may be an important reaction product and has a relatively good stability in natural systems.

**Radiolysis** driven by decay of radioactive minerals can also form additional oxidizers and reducers in the bedrock system. It has been recently demonstrated that in crystalline bedrock environments, sulphate may originate from oxidation of sulphide minerals *in situ*. Oxidative radicals derived from radiolysis of water were found to interact with the sulphide minerals, releasing both hydrogen and sulphate. The process may thus provide both the electron donor and sulphate molecule for sulphate reduction in deep the subsurface (Li *et al.* 2016).

### 3.3 Microbial sulphur oxidation

Syntrophic cooperation between metabolically different organisms is likely crucial for existence of microbial life in deep, oligotrophic aquifer ecosystems (Morris *et al.* 2013; Lau *et al.* 2016). Recent studies show that sulphur oxidizers play a significant role in sulphur cycle in the deep subsurface. These  $\beta$ -proteobacterial genera lineages (*Thauera* and *Thiobacillus*, *Sulfuritalea* and *Sulfuricella* of *Nitrosomonadales*) can use either hydrogen sulphide, elemental sulphur or  $S_2O_3^{2-}$ . Lau *et al.* (2016) reported these from Witwatersand Basin in South Africa, while Sahl *et al.* (2008) detected similar phylotypes from deep granitic mine site in the USA. The significance of these microbial species is shown with network analysis in study of the microbial ecology of Deccan traps, India (deep biosphere hosted by ~65 Myr old Deccan basalt and Archaean granitic basement) (Gupta *et al.* 2018). Oxidation of reduced sulphur species can provide significant energy yield in deep environments (Osburn *et al.* 2014). These organisms also provide a link between sulphur, nitrogen and carbon cycles in the deep subsurface by gaining energy via denitrification while oxidating sulphur, and acting in consortium with anaerobic methane oxidizing archaea (ANME)(Lau *et al.* 2016). The syntrophy between these microbial groups remove thermodynamic hurdles and increase the feasibility of multiple metabolic reactions to occur under the oligotrophic conditions that dominate in the deep subsurface.

### 3.4 Iron as sulphide sink

Iron contributes to the sulphur cycling in the deep subsurface as a sulphide sink. Sulphide can react with Fe(III) bound in minerals forming sulphur and  $Fe^{2+}$ , resulting to excess sulphide precipitating as ferrous sulphide FeS (Bell *et al.* 2018; Johansson *et al.* 2018). Iron reducing bacteria can contribute to this by releasing iron from silicate minerals in granitic rocks, and again the reduced  $Fe^{2+}$  will precipitate with sulphide. In Olkiluoto groundwater, there is an inverse relationship between dissolved sulphide and ferrous iron, implicating the importance of ferrous sulphide precipitation as controlling factor of the occurrence of these ions in the environment. The bentonite barrier system will reduce the transport of sulphide towards the copper canisters via immobilization of sulphide. However, the reduction of ferric iron may pose a risk due to the destabilizing effect of ferrous iron on smectites (Group *et al.* 2005; Bradbury *et al.* 2014; Soltermann 2014; Pedersen *et al.* 2017).

Furthermore, it has been recently shown that sulphate reducing bacteria in a syntrophic consortium with methanogens are able to grow with ferrous sulphate, hydrogen sulphide and  $CO_2$  as their sole substrates and produce pyrite and methane (Thiel *et al.* 2019). For the deep biosphere, this provides proof for a previously speculated link between iron, sulphur and carbon cycles (Holmkvist, Ferdelman and Jørgensen 2011; Thiel *et al.* 2019).

## 4 Availability of electron donors for sulphate reduction

Sulphate reducing microorganisms can be divided into heterotrophic SRP (sulphate reducing prokaryotes) and autotrophic SRP, based on the usage of electron donor. Heterotrophic SRP oxidize organic substrates either incompletely, producing acetate and CO<sub>2</sub> or completely, with CO<sub>2</sub> as a final product. Autotrophic SRP compete of hydrogen with hydrogenotrophic methanogens and acetogens. The values of growth rate, substrate affinity, and substrate threshold explain an order of competitiveness among the three microbial groups at limited H<sub>2</sub> concentrations in which hydrogenotrophic SRP outcompete hydrogenotrophic methanogens and the latter outcompete homoacetogens. When using H<sub>2</sub> as electron donor for sulphate reduction, some seemingly autotrophic sulphate reducers need an additional carbon supply for growth. CO<sub>2</sub> by itself is not enough for the growth, so these microbes need also organic carbon. Therefore, autotrophic SRP are also competing with organotrophs (Canfield, Kristensen and Thamdrup 2005; Liamleam and Annachhatre 2007).

### 4.1 Hydrogen

In bedrock groundwaters, concentration of molecular hydrogen (H<sub>2</sub>) can reach millimolar levels (Figure 3), and a general trend of increasing concentrations with depth has been observed within the Finnish sites included in the deep hydrogeochemical database compiled in this study.

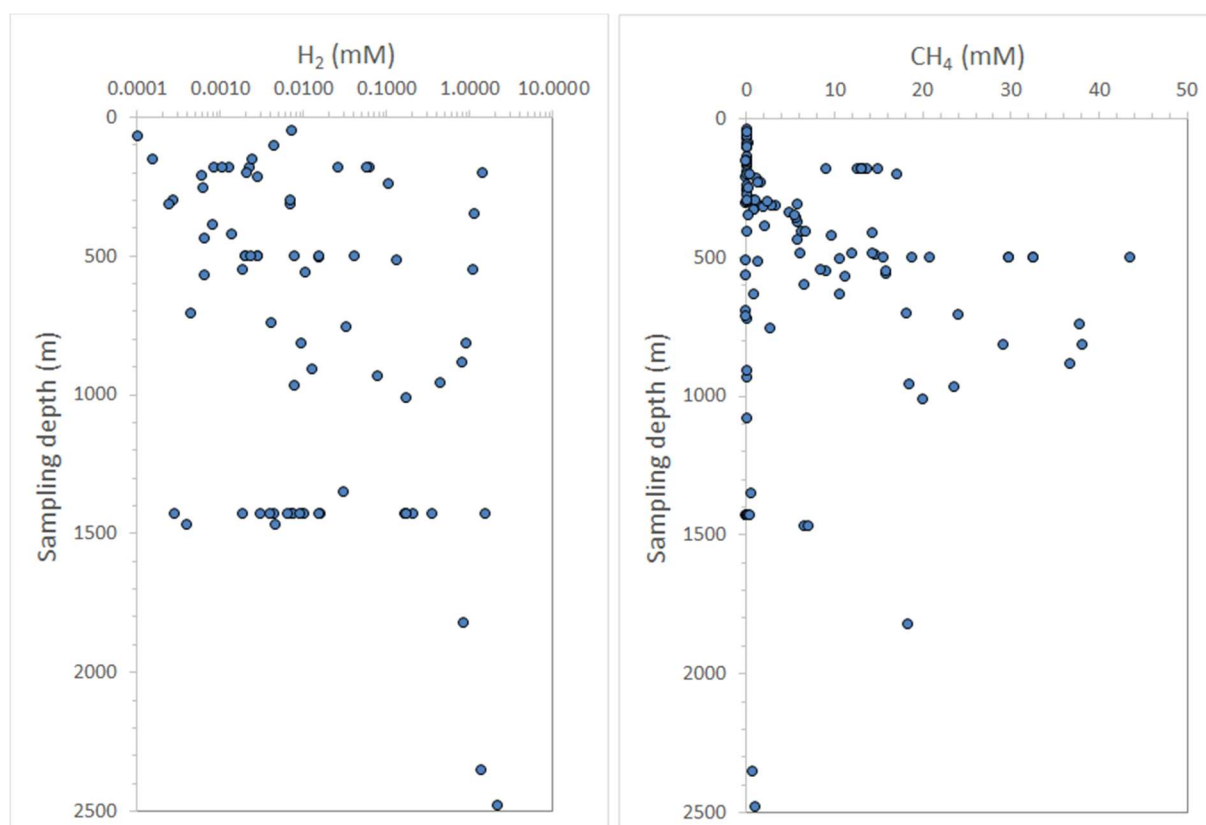
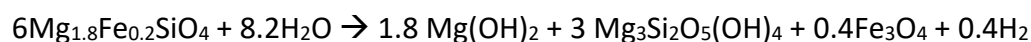


Figure 3. Concentrations of hydrogen and methane with depth in deep bedrock groundwaters in Finland. Data is from the deep groundwater database compiled in the MIND project.



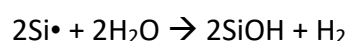
Several processes in the bedrock are known to be hydrogen producing. In radiolytic pathway, water molecules break apart by ionising radiation to form molecular hydrogen, hydrogen radicals and oxidising species, mainly oxygen radicals, which can further react with reduced minerals, such as pyrite (e.g. Lefticariu *et al.* 2010; Dzaugis, Spivack and D'Hondt 2015). Hydrogen and oxygen radicals may also recombine to water molecules, however, molecular hydrogen will be stable and can accumulate. This pathway should be feasible in rocks, such as granite, naturally enriched in U, Th and other radioactive elements, or where radiation from for example radioactive waste is within reach to water. Due to their related formation mechanisms, radiolytic hydrogen may be accompanied with elevated concentrations of other radiogenic nuclides, such as  $^4\text{He}$ .

Hydrogen can also form from reduction of water combined with oxidation of  $\text{Fe}^{2+}$ , a process that can naturally prevail especially in mafic and ultramafic rocks with sufficient Fe-content and mineralogy that is unstable at aqueous, low-temperature environment. The best studied process of this kind is serpentinisation in which olivine hydrolyses to form brucite, serpentine, magnetite and molecular hydrogen (e.g. McCollom and Bach 2009; Mayhew *et al.* 2013):



Likewise, hydrogen can be released in a nuclear waste repository, if iron is used as a canister material and suitable redox conditions are met.

Furthermore, mechanical or mechanochemical pathway is hypothesised based on correlation observed between elevated  $\text{H}_2$  concentrations and seismically active faults and experimental evidence (e.g. Wakita *et al.* 1980; Kita, Marsuo and Wakita 1982; Hirose, Kawagucci and Suzuki 2011; McMahon, Parnell and Blamey 2016). In this pathway, hydrogen is produced from fracture, pore or crystallographic water through frictional grinding. A suggested general reaction is the one where frictionally formed silicon radicals react with water (Kita and Matsuo 1982, McMahon *et al.* 2016):



In addition to seismicity, blasting and grinding of rocks can have the same effect.

Fermentative hydrogen production is common under anoxic conditions. Thus, hydrogen in deep subsurface can also be biologically produced (Nandi and Sengupta 1998; Wang and Wan 2009). The most common hydrogen producers in these surroundings are probably fermentative Clostridia. These are significant part of the microbial community in several deep aquifers as well as serpentinization sites (Brazelton, Nelson and Schrenk 2012; Tiago and Veríssimo 2013; Ben Maamar *et al.* 2015; Purkamo *et al.* 2016a).

## 4.2 Methane

Methane is often the most abundant carbon compound found in bedrock groundwaters, and concentrations of tens of millimoles per litre are not uncommon (Figure 3). Geological sources of methane include thermogenic production, from break-up of organic matter at elevated temperature and pressure, and abiotic production, from catalytic reactions between  $\text{H}_2$  and inorganic carbon. While the former type is globally important in sedimentary formations, thermogenic origin is likely not that common in crystalline rocks, especially granitic rocks devoid of organic carbon sources, and abiotic pathways are suggested to dominate. This is also supported by isotopic evidence, which shows that methane in crystalline rocks is more

enriched in  $^{13}\text{C}$  and relative isotope composition among alkanes is different than in thermogenic methane (e.g. Sherwood Lollar *et al.* 1993b; Etiope and Sherwood Lollar 2013; Kietäväinen *et al.* 2017). At low-temperature conditions ( $<122^\circ\text{C}$ ), microbial methane produced by methanogenic archaea is also commonly encountered especially in the uppermost 1.5 km of the bedrock (Kietäväinen and Purkamo 2015).

As methane is abundantly available in deep crystalline rock environments, we can readily hypothesize its use as an electron donor for sulphate reduction. Sulphate reduction coupled to methane oxidation is usually performed by consortia of sulphate-reducing bacteria and methane-oxidizing ANME archaea. Sulphate acts as the terminal electron acceptor in reaction  $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$  (Knittel and Boetius 2009). ANME archaea form three phylogenetically separate groups, 1-3, and ANME-2 archaea are further divided to lineages a, b, c and d. ANME-1 and ANME-2 archaea are usually associated with sulphate reducers of the *Desulfosarcina-Desulfococcus* branch of the Deltaproteobacteria. In principle, ANME-2 can associate with any bacteria that possess the capacity to scavenge the disulphide produced. ANME-3 archaea are, if at all, associated with *Desulfobulbus* (Knittel and Boetius 2009). Interestingly, Milucka *et al.* (2012) showed that ANME-2 are also able to reduce sulphate to zero-valent sulfur and potentially to sulphide autonomously. The Deltaproteobacteria found in the consortium gain energy by disproportionation of the produced elemental sulfur to sulphide and sulphate (Milucka *et al.* 2012). ANME-2d are capable of independent methane oxidation through reverse methanogenesis using nitrate as the terminal electron acceptor. Nitrite produced by ANME-2d is reduced to dinitrogen gas through a syntrophic relationship with an anaerobic ammonium oxidizing bacterium. These organisms functioning in syntrophic interaction are an important link between carbon and nitrogen cycles (Haroon *et al.* 2013).

ANME have been detected from deep crystalline rock biosphere from several locations worldwide: ANME-1 and ANME-2d from Olkiluoto, the latter shown to belong to the core community at 300-700 m depths (Nyyssönen *et al.* 2012; Bomberg *et al.* 2014, 2015), ANME-2d from Pyhäsalmi mine, Finland (Miettinen *et al.* 2015), an ANME-2d archaeum from Mizunami, Japan, lacking genes for nitrate reduction (Ino *et al.* 2018), ANME-1 from serpentinization-driven subsurface aquifer from Portugal (Tiago and Veríssimo 2013), and ANME from South African Beatrix and Evander mine sites (Gehring *et al.* 2006; Simkus *et al.* 2016). Isotopic evidence of anaerobic oxidation of methane through the history of the Fennoscandian Shield has also been shown (Drake *et al.* 2017). From Outokumpu, however, no ANME archaea have been detected (Purkamo *et al.* 2015, 2016; Rajala *et al.* 2015).

### 4.3 Acetate and other organic compounds

Suitable organic compounds for microbial sulphate reduction found in bedrock groundwater include hydrocarbons, such as alkanes, monocarboxylic acids such as acetate, alcohols such as 2-propanol and 2-butanol and aromatic compounds such as toluene and benzene. Their concentrations in non-contaminated waters are minute, except for hydrocarbons (mainly methane, see 4.1) and in some cases formate and acetate, which can reach concentrations of tens to hundreds of milligrams per litre (Kietäväinen *et al.* 2017; Lamminmäki *et al.* 2017, Kieft *et al.* 2018; Kietäväinen *et al.* in prep.)

Recently, the deep biosphere research has begun to show increasing interest on the role of heterotrophic metabolisms in the subsurface ecosystems. Without a doubt, some part of the deep biosphere is fuelled by hydrogen and carbon dioxide originating from geological origin

(Pedersen 1997), but recent studies have shown that in Fennoscandian bedrock environments, several microbial populations can rely on fermentation of organic carbon and gain energy by nitrate reduction coupled with oxidation of sulphur compounds (Wu *et al.* 2016). In Outokumpu deep subsurface, where sulphate concentrations are generally low, it has been speculated that sulphate reduction exists but that the SRP have to rely on other metabolic strategies like fermentation (Purkamo *et al.* 2015). Dead biomass may provide a source of organic carbon for SRP in deep biosphere. Activity of bacteriophages may result to extensive cell lysis, releasing significant amount of organic compounds for surviving microbial community to utilize (Kyle *et al.* 2008; Pedersen 2012). Dead biomass, i.e. necromass has been shown to contribute to the carbon pool of the deep marine biosphere (Lloyd *et al.* 2013; Lever *et al.* 2015; Bradley, Amend and LaRowe 2018). Thus, it is likely that at least to some extent, necromass can support living biomass in deep terrestrial crystalline rock environments as well.

#### **4.4 Release from rock matrix**

Compared to groundwater, the amount and concentration of gases in the host rocks have not been widely reported. However, recently the amount of hydrogen and methane release was calculated from Onkalo drill hole cores (Kietäväinen *et al.* 2018 -Mind 2.1.2 report). The amount of gases increased within the depth, and concentrations were on average 18mL/L of H<sub>2</sub> and 31 mL/L of CH<sub>4</sub> in the core porewater.

## **5 Essential nutrients**

Nitrogen, phosphorus and sulphur are essential elements for life. Nitrogen is needed for the synthesis of amino and nucleic acids, while phosphorus is a building block of nucleic acids, nucleotides, phosphoproteins and phospholipids. Phosphorus is vital to energy production and energy storage in cells. Sulphur is a building block of certain amino acids. Cells acquire sulphur for biosynthesis via assimilatory sulphate reduction. These nutrients may represent limiting elements for microbial growth in deep subsurface groundwater. Iron and other trace elements are important micronutrients for microbes, as those are essential components of many enzymes (Schröder, Johnson and De Vries 2003). P, N and Fe are the most common limiting nutrients in almost all ecosystems (Lever *et al.* 2015 and references within). Microbes can scavenge elemental nutrients by producing protons to enhance mineral dissolution, iron chelation or reductive dissolution to release bound elements (Rogers and Bennett 2004). Microbial communities in oligotrophic environments have developed strategies to survive in these habitats. Syntrophic cooperation will provide maximum energy yield and facilitate growth (Lau *et al.* 2016). Dead biomass can support the maintenance of substantial amount of cells (up to thousands) in low-energy environments, such as deep marine sediments (Bradley, Amend and LaRowe 2018) and hypothetically also in deep continental groundwaters.

### **5.1 Nitrogen**

Nitrogen is a key element in the structure of amino acids, proteins, nucleic acids, and other molecules essential to life. Nitrogen is also the dominant component of Earth's atmosphere, from where it is incorporated into the biosphere via nitrogen fixation. Specialized microbes use nitrogenase enzymes in this energy-hungry process requiring 16 ATP molecules to break the triple bond of N<sub>2</sub>. Nitrogen fixation is shown to have evolved very early in life's history, as the abiotic sources of nitrogen have been scarce (Zerkle and Mikhail 2017 and references

within). Microbes in deep Fennoscandian subsurface have the capacity to fix nitrogen, shown by metagenomics (Nyyssönen *et al.* 2014; Wu *et al.* 2016). Nitrogen fixing ability has also been shown by the detection of *nifH* marker gene from other deep subsurface sites (Swanner and Templeton 2011; Lau *et al.* 2016).

Nitrogen, in the form  $N_2$ , is introduced to groundwaters during recharge both in contact with atmospheric air and in the undersaturated zone above the water table. As for other gases, solubility of  $N_2$  is controlled by temperature and pressure such that more  $N_2$  can dissolve in water in cold than in warm conditions and under increased pressure. However, concentration of nitrogen in bedrock groundwaters typically exceeds that of air saturated water (e.g. Silver *et al.* 2012). Some nitrogen is also formed as a result of the radioactive decay of  $^{14}C$  with a half-life of 5730 years, and due to water-rock interaction within the bedrock.

$N_2$  is present in all groundwaters, including the deep and saline ones, but concentrations or presence of other nitrogen forms, mainly  $NO_3^-$  and  $NH_3$ , are reported more rarely, or their concentrations have been below detection limit. From the deep groundwater database collected in this study, it can be concluded that  $N_2$ -dominated groundwaters typically have lower total gas content than methane-dominated groundwaters.

Few studies have reported isotopic composition of  $N_2$  in deep continental groundwaters, with  $\delta^{15}N$  values between -1.7 ‰ and 10 ‰ relative to the atmospheric ratio, and mostly in line with isotopic composition of nitrogen in meteoric waters, and thus atmospheric origin, with the lowest and highest values possibly related to additional water-rock interaction and microbial transformations (Sherwood Lollar *et al.* 1993a; Möller *et al.* 1997; Pitkänen and Partamies 2007; Silver *et al.* 2012).

Although atmosphere on Earth is usually thought to be the explicit source of nitrogen for life, the nitrogen reservoir of the continental crust ( $1.7 \times 10^{18}$  kg) is in the same scale as the atmosphere ( $4.0 \times 10^{18}$  kg) (Johnson and Goldblatt 2015). Geologic nitrogen has been shown to be a significant source of nitrogen in some terrestrial and aquatic ecosystems. Nitrogen occurs in sedimentary, metamorphic, and igneous rocks with concentrations ranging from a few mg of N to >10 g of N per kg. Precambrian biotites obtained from Finnish bedrock contain 20-40 mg of N/kg (Itihara and Suwa 1985; Holloway and Dahlgren 2002; Papineau *et al.* 2005). Phyllosilicates such as micas are abundant in Outokumpu (Västi 2011), thus these can provide an abiotic source of nitrogen for the deep biosphere (Lau *et al.* 2016).

## 5.2 Phosphorus

Apatite is a common accessory mineral and usually the main host of phosphorus in crystalline bedrock. A large number of microbial organisms including bacteria, fungi, and algae exhibit P solubilization and mineralization ability. Microbes produce phosphatase enzymes to cleave phosphate from organic compounds. Furthermore, microbial weathering of phosphate-containing minerals has been demonstrated (Taunton, Welch and Banfield 2000; Welch, Taunton and Banfield 2002). In Outokumpu, measured phosphorus range is highly variable, and microbes uptake phosphate through organic and inorganic mineralization processes (Nyyssönen *et al.* 2014).

In oligotrophic environments such as the deep continental subsurface, the ability to scavenge available phosphorus from minerals is crucial. Preferential colonization of certain rocks containing trace phosphorus as apatite inclusions has been detected when P concentrations are low in groundwater (Bennett *et al.* 2001). Phosphorus bound to silicate minerals is

bioavailable: microbes use organic ligands to dissolve the silicate matrix in order to access to nutrients. The access strategies microbes use include the production of protons to enhance silicate matrix dissolution, iron chelation or reductive dissolution (Rogers and Bennett 2004). The microbiologically driven weathering of granitic rock minerals has also been experimentally shown (Abdulla 2009). A microbial consortium of *Actinomyces*-affiliating microbes had potentially multiple roles in weathering of granitic rocks by acid production, metal leaching and solubilisation of phosphate and sulphate in the cultures (Abdulla 2009).

## 6 Transport of matter as a limiting factor

All components required for the sulphide production reaction must be available within a certain range, allowing replenishment of the consumed reactants. Considering the detrimental role of dissolved sulphide in the repository near field, we may divide the migration process in two scenarios:

- 1) sulphide migration from the surrounding bedrock to the near field
- 2) migration of all components required for microbial sulphate reduction to the near field

For the latter case, components required can be divided in two categories: those recycled by the microbes and those consumed in the reaction, thus requiring a continuous supply.

Continuous supply by transport is potentially important with respect to sulphur compounds (mainly sulphide and/or sulphate) and electron donors. Sulphide is a common dissolved component in deep reducing groundwaters at concentration levels 0.01 mM (Fig. 3), whereas sulphate concentrations in the uppermost groundwaters (down to depth 500 m) of the crystalline rock may be as high as 5 mM, but typically decreases drastically below a certain level. Methane, a potential electron donor may be available at concentration levels up to 50 mM. In addition to sulphur sources in situ, sulphate can migrate in the repository from the outside (sea water, surface water etc.). Dissolved gases may originate from different sources, either migrated even from deep sources or formed in situ. Essential nutrients and micronutrients of deep life are available either in groundwaters or in rock forming minerals. Demand of nutrients may, however, be compensated by recycling.

### 6.1 General model

Following generic transport model of these components do not discriminate with the different transport properties of different solutes considered (i.e.  $\text{SO}_4^{2-}$ ,  $\text{HS}^-$ ,  $\text{CH}_4$  etc.). Neither is the geometry of the system aimed to be representative of any actual site or disposal concept. Possible complex coupling between processes is also neglected, and the aim is entirely to estimate the order-of-magnitude transport characteristics with respect to sulphide production.

The generic near-field diffusion is taken to have spherical geometry: outer surface of the deposition hole containing a metal canister embedded in bentonite forms a spherical surface with a radius of 1.5 m. This surface ( $\approx 28 \text{ m}^2$ ) is assumed to be the active near-field zone in contact with the bentonite buffer. Diameter of the entire spherical diffusion model is 30 m ( $r=15 \text{ m}$ ). Diffusion coefficient of the considered solutes ( $\text{HS}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{CH}_4$ ) in water ( $D_w$ ) are taken to be  $1 \cdot 10^{-9} \text{ m}^2/\text{s}$ . Porosity ( $\phi$ ) available for diffusion was assumed to be 0.01 (1%). Effective diffusion coefficient in the porous bulk rock would thus be about  $1 \cdot 10^{-11} \text{ m}^2/\text{s}$

( $De=D_w \cdot \phi$ ), if factors as tortuosity (e.g. Shen and Chen 2007) and sorption/retardation are neglected. Concentration of the solute considered was assumed to be 1 mM (1 mmol/L in porewater). Diffusion model was based on the porous-continuum approach, implying that the solid and the fluid phases constitute a single medium, over which the diffusion equation applies. Consequently, the initial concentration of the diffusive component in the medium is  $1 \cdot 10^{-5} \text{ mol/m}^3$  of rock.

The model was calculated numerically as a function of time using Comsol Multiphysics. At the time  $t=0$ , the solute was evenly distributed in the rock surrounding the spherical “deposition hole” forming a sink to the solute (i.e. a zero concentration boundary). Figure 4 shows the evolution of the concentration around the sink during the first 10 Ka, indicating that the effect of diffusion restricts to the distance of about 6 m from the sink. Figure 5 shows the solute flux rate to the surface of the “deposition hole”.

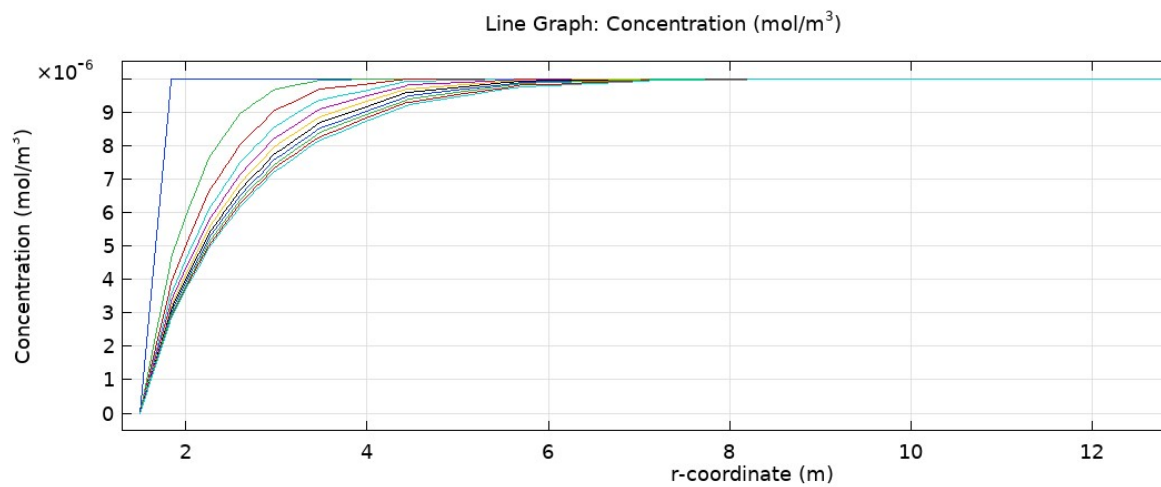


Figure 4. Evolution of the concentration gradient as a function of the distance and time: curves from left to right correspond to subsequent time steps of 1 ka from 0 to 10 ka.

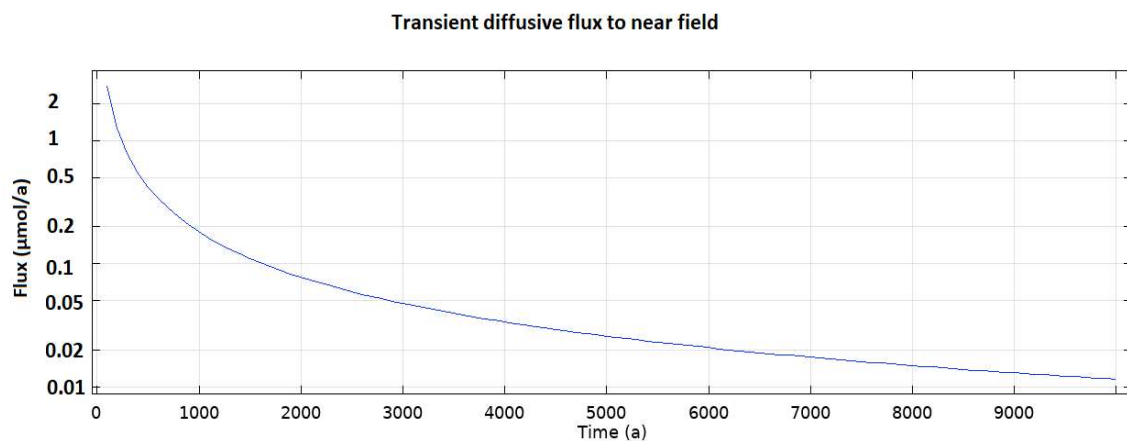


Figure 5. Diffusive flux to the “deposition hole” as a function of time.

The diffusion model is transient, because it is not explicitly coupled to a possible advective transport supply that would compensate for the consumption of the solute. This coupling is done here qualitatively, and visualized in the Figure 6. We assume average hydraulic conductivity for the rock mass,  $k = 10^{-9}$  m/s, and gradient 0.1 m/m (clearly conservative estimates), giving a darcian velocity vector  $1 \cdot 10^{-10}$  m/s (3 mm/a). Taking into account the “effective porous concentration” of the solute,  $1 \cdot 10^{-5}$  mol/m<sup>3</sup>, we can estimate the advective supply to the cross section area of the diffusion model sphere (2800 m<sup>2</sup>) to be 88  $\mu$ mol/a.

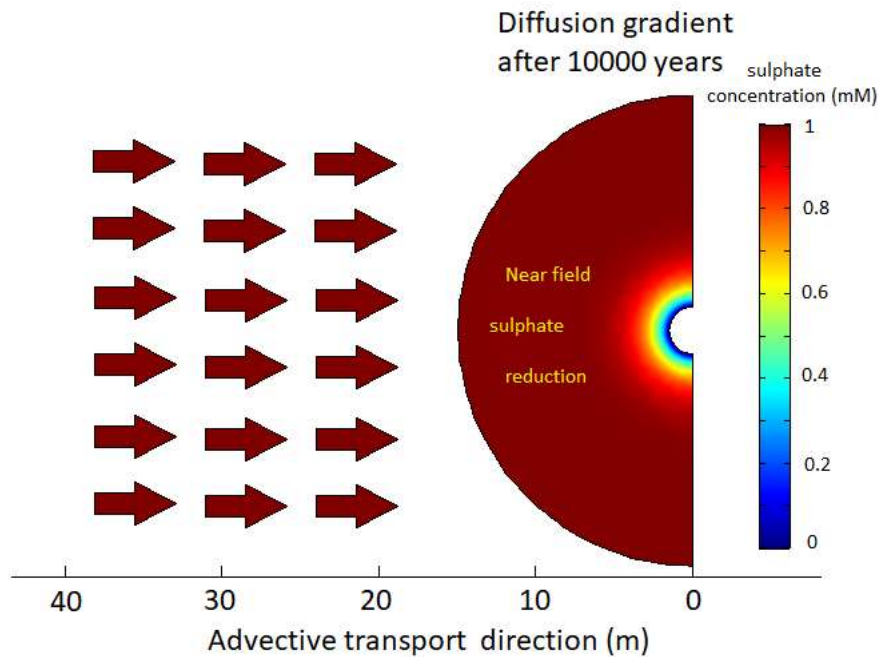
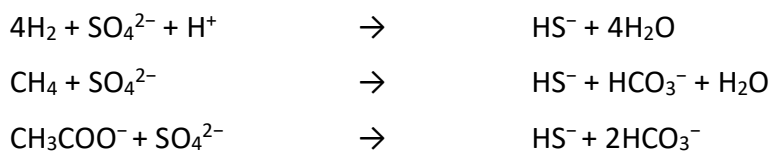


Figure 6. Qualitatively coupled advection-diffusion model to estimate the supply of reactants for microbial sulphate reduction.

Reaction between metallic copper and sulphide has the stoichiometry 2:1 in anoxic conditions, leading mainly to the formation of chalcocite ( $\text{Cu}_2\text{S}$ ), whereas sulphidization of iron produces iron monosulphide (e.g. mackinawite). Depending on the electron donor (hydrogen/methane/acetate), stoichiometry of the microbial sulphide formation from sulphate may be expressed as:



Evidently the mass transport constraint is a very effective limitation of the microbial sulphidic corrosion of metals. These calculations indicate maximum overall corrosion rates of the order of micromoles of copper per year and per deposition hole in the KBS-3 concept.

## 7 Thermodynamic constraints on sulphide formation and accumulation

Microbes harvest energy via oxidation-reduction processes. This energy is usually converted to high-energy compounds such as ATP (adenosine triphosphate) that cells can then use to drive energetically more unfavourable reactions. The minimum amount of energy required to form one mole of ATP under normal cellular conditions is  $\sim 50$  kJ. This is commonly used as a proxy for microbial growth (LaRowe, Dale and Regnier 2008). The minimum energy required for ATP synthesis can be even lower than  $-10$  kJ/mol with ion gradient-driven phosphorylation (Müller and Hess 2017). In-situ investigations of a limiting threshold for active populations is 190 zeptoWatts per cell in extreme oligotrophic environments. Values for the minimum microbial cell maintenance power are theoretically as low as 1 zeptoWatt/cell (LaRowe and Amend 2015). Anaerobic oxidation of methane coupled with sulphate reduction has the lowest free energy yield among possible electron acceptors. ANME coupling methane oxidation with nitrate or nitrite reduction gain more energy, so nitrate-coupled AOM is energetically superior (Caldwell *et al.* 2008).

Typical concentrations of sulphate and sulphide detected in deep bedrock groundwaters in Finland are shown in Figure 7.

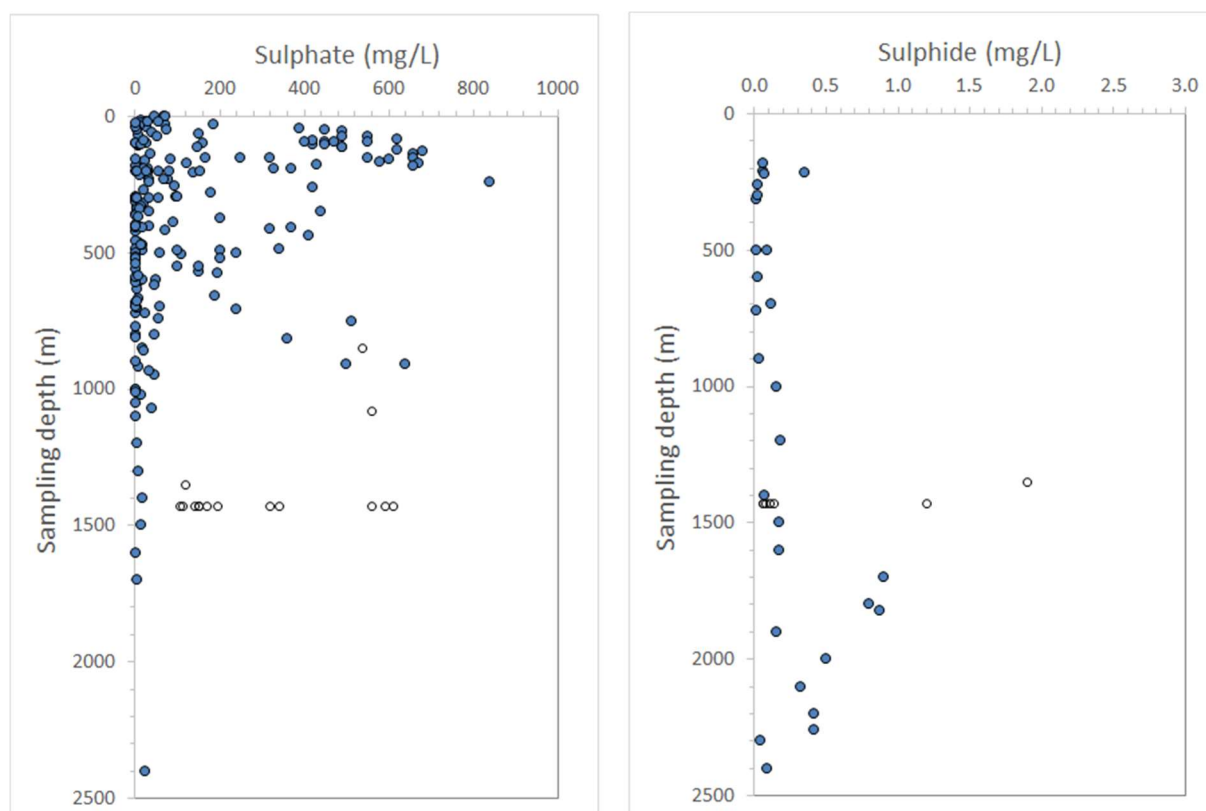


Figure 7. Concentrations of sulphate and sulphide with depth in deep bedrock groundwaters in Finland. Open circles = Pyhäsalmi mine. Data is from the deep groundwater database compiled in the MIND project.



Thermodynamic calculations here for sulphate reduction reactions were done according to LaRowe and Amend (2014) and Osburn *et al.* (2014). First, Gibbs free energies of reactions ( $\Delta G_r$ ) were calculated at *in situ* temperature and *pe* which was modelled using PHREEQC (USGS) based on both C(-4)/C(4) and H(0)/H(1) redox couples determined from the laboratory analysis results from two sites (Outokumpu Deep Drill Hole and Pyhäsalmi mine). Based on the  $\Delta G_r$ , stoichiometry ( $\nu_i$ ) and concentration of the limiting species [ $i$ ] of each reaction, energy densities ( $E_r$ ) were calculated using the equation:

$$E_r = |\Delta G_r / \nu_i| [i]$$

The limiting species can be either electron acceptor or donor. Due to the high rock/water ratio, and following the practice of LaRowe and Amend (2014), solids were not considered to be limiting.

Typical energy densities were below 0.4 J/L for the  $\text{SO}_4 + \text{H}_2$  reaction and below 0.1 J/L for the  $\text{SO}_4 + \text{CH}_4$  reaction, except at one drill hole at Pyhäsalmi (R2250) where the energy density of the  $\text{SO}_4 + \text{CH}_4$  reaction was 0.56 J/L (Figure 8). At Outokumpu sulphide production seems to be mostly limited by the availability of sulphate, while availability of electron donors is more important control at Pyhäsalmi.

Adding time into the equation, energies can also be expressed as Watts per litre. By choosing a relevant timescale of 100 kiloyears, power available from the sulphate reduction reactions is 1 - 100 fW/L ( $10^{-15}$  to  $10^{-13}$  W/L) which is suggested to sustain  $10^8$  microbial cells/L at most, within the considered timeframe and the minimum maintenance power for one microbial cell ( $10^{-21}$  W) (LaRowe and Amend 2015).

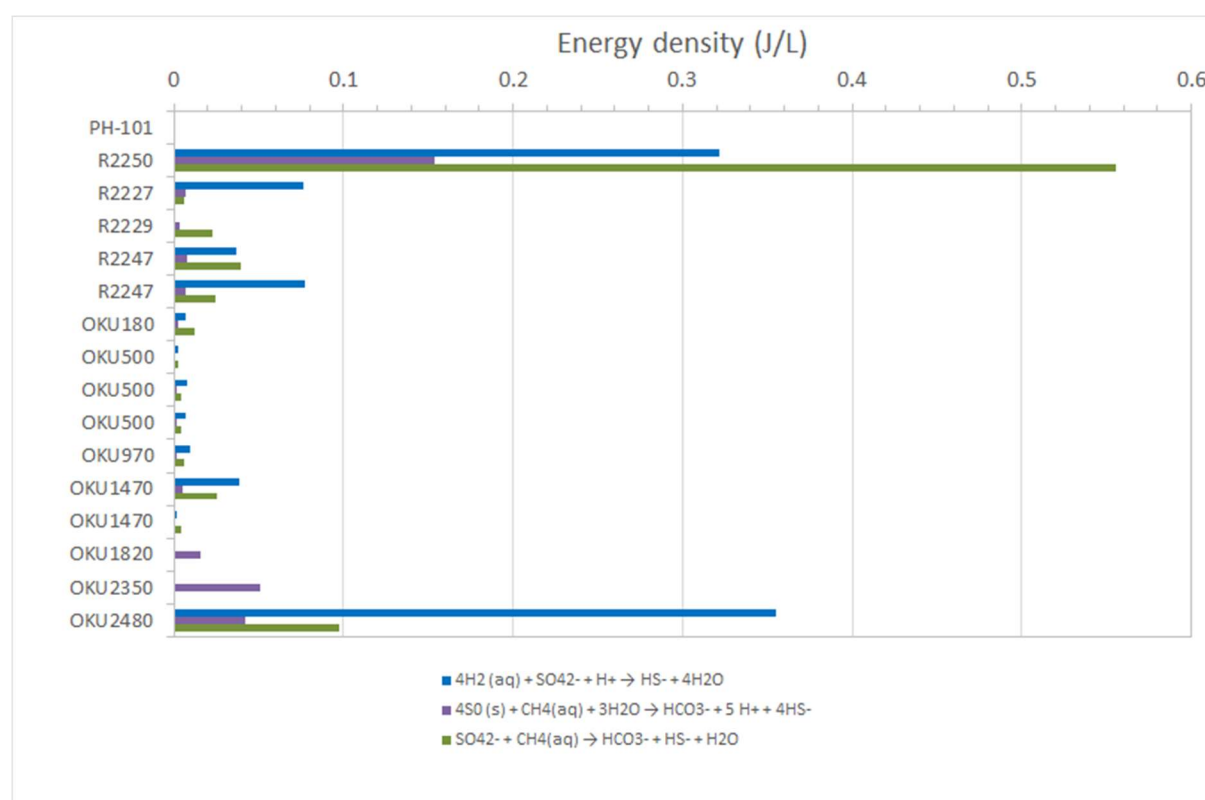


Figure 8. Energy densities of sulphide producing reactions in the Pyhäsalmi mine (samples PH-101 – R-2247) and Outokumpu Deep Drill Hole (samples OKU180 – OKU2480).

## 8 Conclusions

We have identified and studied following factors possibly controlling microbial sulphide production in the geosphere:

- redox potential
- solubility limitation
- sulphur availability limitation
- electron donor limitation
- nutrient limitation
- transport limitation
- energy limitation

Redox conditions of the groundwater-bedrock system can be considered as a qualitative measure of the sulphide production capacity of the system. High redox potential indicating the presence of oxygen or other strong electron acceptors suppresses effectively sulphate reduction to sulphide. Measured redox potentials of natural groundwater system must be interpreted with care, because disequilibrium between different redox pairs is common.

Sulphide solubility limitation together with a mass transport constraint is a straightforward way used in safety case to estimate canister corrosion. However, rapid sulphate reduction may lead to the deficiency of iron or other component required for the precipitation.

Even though sulphur-bearing minerals are not always common in all rock types, as for example in granitic rocks, presence of mobile sulphur compounds can hardly be excluded in any disposal system.

Presence of electron donors in sufficient amounts is one of the most important controlling factors for sulphide formation. Dissolved molecular hydrogen is the most effective electron donor, whereas methane is the most common one. Currently, more attention has been paid to the role of organic C-2 compounds, especially acetate.

Nutrient limitation is not considered as an important limiting factor, because the bedrock environment provides an adequate source. In deep biosphere, recycling of essential elements and syntrophic interactions play key roles in sustaining microbial life. Metagenomic studies have established the ability of microbial nitrogen fixation. Microbes have different strategies to contribute to mineralization processes. Current “steady state” of microbial population may be disturbed by e.g. underground construction work. Aeration of bedrock environments will increase the abundance of aerobic organisms and change the dynamics of sulphate cycling.

Transport limitations, based on advection and diffusion, seems to be an easily quantified way to estimate long-term sulphide production rates. Ideal conditions in terms of all other factors required by the microbial sulphide production would evidently lead to limited supply by the limited transport rate.

Energetic calculations demonstrate that life deep in the bedrock has a plausible energy source supplied by the surrounding chemical energy. The life-sustaining energy flux, i.e. power, remains however low, and cannot be multiplied by many orders of magnitude in a sustainable way.

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## 10 References

- Abdulla H. Bioweathering and biotransformation of granitic rock minerals by actinomycetes. *Microb Ecol* 2009, DOI: 10.1007/s00248-009-9549-1.
- Bell E, Lamminmäki T, Alneberg J *et al.* Biogeochemical cycling by a low-diversity microbial community in deep groundwater. *Front Microbiol* 2018, DOI: 10.3389/fmicb.2018.02129.
- Bennett PC, Rogers JR, Choi WJ *et al.* Silicates, silicate weathering, and microbial ecology. *Geomicrobiol J* 2001;**18**:3–19.
- Bethke CM, Sanford R a., Kirk MF *et al.* The thermodynamic ladder in geomicrobiology. *Am J Sci* 2011;**311**:183–210.
- Bomberg M, Nyssönen M, Nousiainen A *et al.* Evaluation of Molecular Techniques in Characterization of Deep Terrestrial Biosphere. *Open J Ecol* 2014;**04**:468–87.
- Bomberg M, Nyssönen M, Pitkänen P *et al.* Active Microbial Communities Inhabit Sulphate-Methane Interphase in Deep Bedrock Fracture Fluids in Olkiluoto, Finland. *Biomed Res Int* 2015;**2015**.
- Bradbury MH, Berner U, Curti E *et al.* *The Long Term Geochemical Evolution of the Nearfield of the HLW Repository.*, 2014.
- Bradley JA, Amend JP, LaRowe DE. Necromass as a Limited Source of Energy for Microorganisms in Marine Sediments. *J Geophys Res Biogeosciences* 2018, DOI: 10.1002/2017JG004186.
- Brazelton WJ, Nelson B, Schrenk MO. Metagenomic evidence for H<sub>2</sub> oxidation and H<sub>2</sub> production by serpentinite-hosted subsurface microbial communities. *Front Microbiol* 2012;**2**:1–16.
- Caldwell SL, Laidler JR, Brewer E a. *et al.* Anaerobic oxidation of methane: Mechanisms, bioenergetics, and the ecology of associated microorganisms. *Environ Sci Technol* 2008;**42**:6791–9.
- Canfield DE, Kristensen E, Thamdrup B. Heterotrophic Carbon Metabolism. *Advances in Marine Biology*. Vol 48. 2005, 129–62.
- Drake H, Heim C, Roberts NMW *et al.* Isotopic evidence for microbial production and consumption of methane in the upper continental crust throughout the Phanerozoic eon. *Earth Planet Sci Lett* 2017;**470**:108–18.
- Dzaugis ME, Spivack AJ, D'Hondt S. A quantitative model of water radiolysis and chemical production rates near radionuclide-containing solids. *Radiat Phys Chem* 2015;**115**:127–34.
- Etiopie G, Sherwood Lollar B. Abiotic methane on Earth. *Rev Geophys* 2013;**51**:276–99.
- Gihring TM, Moser DP, Lin L-HHL-H *et al.* The distribution of microbial taxa in the subsurface water of the Kalahari Shield, South Africa. *Geomicrobiol J* 2006;**23**:415–30.
- Group EG, Ge M, Paul S *et al.* Experimental study of smectite interaction with metal Fe at low temperature: 1. Smectite destabilization. *Clays Clay Miner* 2005;**53**:597–612.
- Gupta A, Dutta A, Sar P *et al.* Exploration of deep terrestrial subsurface microbiome in Late Cretaceous Deccan traps and underlying Archean basement, India. *Sci Rep* 2018;**8**:17459.
- Haroon MF, Hu S, Shi Y *et al.* Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* 2013;**500**:567–70.
- Hirose T, Kawagucci S, Suzuki K. Mechanoradical H<sub>2</sub> generation during simulated faulting: Implications for an earthquake-driven subsurface biosphere. *Geophys Res Lett* 2011;**38**, DOI: 10.1029/2011GL048850.
- Holloway JM, Dahlgren RA. Nitrogen in rock: Occurrences and biogeochemical implications. *Global Biogeochem Cycles* 2002, DOI: 10.1029/2002gb001862.
- Holmkvist L, Ferdelman TG, Jørgensen BB. A cryptic sulfur cycle driven by iron in the methane zone of

- marine sediment (Aarhus Bay, Denmark). *Geochim Cosmochim Acta* 2011, DOI: 10.1016/j.gca.2011.03.033.
- Ino K, Hermsdorf AW, Konno U *et al.* Ecological and genomic profiling of anaerobic methane-oxidizing archaea in a deep granitic environment. *ISME J* 2018;**12**:31–47.
- Itihara Y, Suwa K. Ammonium contents of biotites from Precambrian rocks in Finland: The significance of NH<sub>4</sub><sup>+</sup> as a possible chemical fossil. *Geochim Cosmochim Acta* 1985, DOI: 10.1016/0016-7037(85)90198-X.
- Johansson L, Stahlén J, Taborowski T *et al.* Bacterial release of iron from Olkiluoto rock minerals, WR2018-30. 2018;**31**.
- Johnson B, Goldblatt C. The nitrogen budget of Earth. *Earth-Science Rev* 2015, DOI: 10.1016/j.earscirev.2015.05.006.
- Kieft TL, Walters CC, Higgins MB *et al.* Dissolved organic matter compositions in 0.6–3.4 km deep fracture waters, Kaapvaal Craton, South Africa. *Org Geochem* 2018, DOI: 10.1016/j.orggeochem.2018.02.003.
- Kietäväinen R, Ahonen L, Niinikoski P *et al.* Abiotic and biotic controls on methane formation down to 2.5 km depth within the Precambrian Fennoscandian Shield. *Geochim Cosmochim Acta* 2017;**202**:124–45.
- Kietäväinen R, Purkamo L. The origin, source and cycling of methane in deep crystalline rock biosphere. *Front Microbiol* 2015;**6**:725.
- Kietäväinen R, Ahonen L, Pedersen K. 2017. Interim report on deep gases and sulphur compounds as biogeochemical energy sources in crystalline rock. MIND Deliverable 2.3., 19 pages
- Kita I, Marsuo S, Wakita H. H<sub>2</sub> Generation By Reaction Between H<sub>2</sub>O and Crushed Rock: an Experimental Study on H<sub>2</sub> Degassing From the Active Fault Zone. *J Geophys Res* 1982;**87**:10789–95.
- Knittel K, Boetius A. Anaerobic oxidation of methane: progress with an unknown process. *Annu Rev Microbiol* 2009;**63**:311–34.
- Kyle JE, Eydal HSC, Ferris FG *et al.* Viruses in granitic groundwater from 69 to 450 m depth of the spö hard rock laboratory, Sweden. *ISME J* 2008;**2**:571–4.
- LaRowe DE, Amend JP. Microbial Life of the Deep Biosphere. *Microbial Life of Deep Biosphere*. 2014.
- LaRowe DE, Amend JP. Power limits for microbial life. *Front Microbiol* 2015;**6**:1–11.
- LaRowe DE, Dale AW, Regnier P. A thermodynamic analysis of the anaerobic oxidation of methane in marine sediments. *Geobiology* 2008;**6**:436–49.
- Lau MCY, Kieft TL, Kuloyo O *et al.* An oligotrophic deep-subsurface community dependent on syntrophy is dominated by sulfur-driven autotrophic denitrifiers. *Proc Natl Acad Sci* 2016;**113**:E7927–36.
- Lefticariu L, Pratt LA, LaVerne JA *et al.* Anoxic pyrite oxidation by water radiolysis products - A potential source of biosustaining energy. *Earth Planet Sci Lett* 2010;**292**:57–67.
- Lever MA, Rogers KL, Lloyd KG *et al.* Life under extreme energy limitation: A synthesis of laboratory- and field-based investigations. *FEMS Microbiol Rev* 2015;**39**:688–728.
- Li L, Wing BA, Bui TH *et al.* Sulfur mass-independent fractionation in subsurface fracture waters indicates a long-standing sulfur cycle in Precambrian rocks. *Nat Commun* 2016:13252.
- Liamleam W, Annachhatre AP. Electron donors for biological sulfate reduction. *Biotechnol Adv* 2007;**25**:452–63.
- Lloyd KG, Schreiber L, Petersen DG *et al.* Predominant archaea in marine sediments degrade detrital proteins. *Nature* 2013;**496**:215–8.
- Ben Maamar S, Aquilina L, Quaiser A *et al.* Groundwater isolation governs chemistry and microbial community structure along hydrologic flowpaths. *Front Microbiol* 2015;**6**:1–13.
- Mayhew LE, Ellison ET, McCollom TM *et al.* Hydrogen generation from low-temperature water-rock reactions. *Nat Geosci* 2013;**6**:478–84.
- McCollom TM, Bach W. Thermodynamic constraints on hydrogen generation during serpentinization of ultramafic rocks. *Geochim Cosmochim Acta* 2009;**73**:856–75.
- McMahon S, Parnell J, Blamey NJF. Evidence for Seismogenic Hydrogen Gas, a Potential Microbial Energy Source on Earth and Mars. *Astrobiology* 2016;**16**:690–702.

- Miettinen H, Kietäväinen R, Sohlberg E *et al.* Microbiome composition and geochemical characteristics of deep subsurface high-pressure environment, Pyhäsalmi mine Finland. *Front Microbiol* 2015;**6**:1203.
- Milucka J, Ferdelman TG, Polerecky L *et al.* Zero-valent sulphur is a key intermediate in marine methane oxidation. *Nature* 2012;**491**:541–6.
- Möller P, Weise SM, Althaus E *et al.* Paleofluids and Recent fluids in the upper continental crust: Results from the German Continental Deep Drilling Program (KTB). *J Geophys Res Solid Earth* 1997;**102**:18233–54.
- Morris BEL, Henneberger R, Huber H *et al.* Microbial syntrophy: Interaction for the common good. *FEMS Microbiol Rev* 2013, DOI: 10.1111/1574-6976.12019.
- Müller V, Hess V. The minimum biological energy quantum. *Front Microbiol* 2017, DOI: 10.3389/fmicb.2017.02019.
- Nandi R, Sengupta S. Microbial production of hydrogen: an overview. *Crit Rev Microbiol* 1998;**24**:61–84.
- Nyyssönen M, Bomberg M, Kapanen A *et al.* Methanogenic and sulphate-reducing microbial communities in deep groundwater of crystalline rock fractures in Olkiluoto, Finland. *Geomicrobiol J* 2012;**29**:863–78.
- Nyyssönen M, Hultman J, Ahonen L *et al.* Taxonomically and functionally diverse microbial communities in deep crystalline rocks of the Fennoscandian shield. *ISME J* 2014;**8**:126–138.
- Osburn MR, LaRowe DE, Momper LM *et al.* Chemolithotrophy in the continental deep subsurface: Sanford Underground Research Facility (SURF), USA. *Front Microbiol* 2014;**5**.
- Papineau D, Mojzsis SJ, Karhu JA *et al.* Nitrogen isotopic composition of ammoniated phyllosilicates: Case studies from Precambrian metamorphosed sedimentary rocks. *Chem Geol* 2005, DOI: 10.1016/j.chemgeo.2004.10.009.
- Pedersen K. Microbial life in deep granitic rock. *FEMS Microbiol Rev* 1997;**20**:399–414.
- Pedersen K. Metabolic activity of subterranean microbial communities in deep granitic groundwater supplemented with methane and H<sub>2</sub>. *ISME J* 2012;**7**:839–49.
- Pedersen K, Bengtsson A, Blom A *et al.* Mobility and reactivity of sulphide in bentonite clays – Implications for engineered bentonite barriers in geological repositories for radioactive wastes. *Appl Clay Sci* 2017;**146**:495–502.
- Pitkänen P, Partamies S. *Origin and Implications of Dissolved Gases in Groundwater at Olkiluoto.*, 2007.
- Purkamo L, Bomberg M, Kietäväinen R *et al.* Microbial co-occurrence patterns in deep Precambrian bedrock fracture fluids. *Biogeosciences* 2016;**13**:3091–108.
- Purkamo L, Bomberg M, Nyyssönen M *et al.* Heterotrophic Communities Supplied by Ancient Organic Carbon Predominate in Deep Fennoscandian Bedrock Fluids. *Microb Ecol* 2015;**69**:319–32.
- Rajala P, Bomberg M, Kietäväinen R *et al.* Rapid Reactivation of Deep Subsurface Microbes in the Presence of C-1 Compounds. *Microorganisms* 2015;**3**:17–33.
- Rogers JR, Bennett PC. Mineral stimulation of subsurface microorganisms: Release of limiting nutrients from silicates. *Chem Geol* 2004;**203**:91–108.
- Sahl JW, Schmidt R, Swanner ED *et al.* Subsurface microbial diversity in deep-granitic-fracture water in Colorado. *Appl Environ Microbiol* 2008;**74**:143–52.
- Schröder I, Johnson E, De Vries S. Microbial ferric iron reductases. *FEMS Microbiol Rev* 2003, DOI: 10.1016/S0168-6445(03)00043-3.
- Shen L, Chen Z. Critical review of the impact of tortuosity on diffusion. *Chem Eng Sci* 2007, DOI: 10.1016/j.ces.2007.03.041.
- Sherwood Lollar B, Frape SK, Fritz P *et al.* Evidence for bacterially generated hydrocarbon gas in Canadian shield and fennoscandian shield rocks. *Geochim Cosmochim Acta* 1993a, DOI: 10.1016/0016-7037(93)90609-Z.
- Sherwood Lollar B, Frape SK, Weise SMM *et al.* Abiogenic methanogenesis in crystalline rocks. *Geochim Cosmochim Acta* 1993b;**57**:5087–97.
- Silver BJ, Raymond R, Sigman DM *et al.* The origin of NO<sub>3</sub><sup>-</sup> and N<sub>2</sub> in deep subsurface fracture water of South Africa. *Chem Geol* 2012;**294–295**:51–62.

- Simkus DN, Slater GF, Lollar BS *et al.* Variations in microbial carbon sources and cycling in the deep continental subsurface. *Geochim Cosmochim Acta* 2016;**173**:264–83.
- Soltermann D. Ferrous iron uptake mechanism at the montmorillonite-water interface under anoxic and electrochemically reduced conditions. *ETH Zurich* 2014, DOI: 10.3929/ethz-a-010239638.
- Swanner E, Templeton A. Potential for nitrogen fixation and nitrification in the granite-hosted subsurface at Henderson Mine, CO. *Front Microbiol* 2011;**2**:254.
- Taunton AE, Welch SA, Banfield JF. Microbial controls on phosphate and lanthanide distributions during granite weathering and soil formation. *Chemical Geology*. 2000.
- Thiel J, Byrne JM, Kappler A *et al.* Pyrite formation from FeS and H<sub>2</sub>S is mediated through microbial redox activity. *Proc Natl Acad Sci* 2019:201814412.
- Tiago I, Veríssimo A. Microbial and functional diversity of a subterrestrial high pH groundwater associated to serpentinization. *Environ Microbiol* 2013;**15**:1687–706.
- Västi K. Petrology of the drill hole R2500 at Outokumpu, eastern Finland—the deepest drill hole ever drilled in Finland. *Geol Surv Finland, Spec Pap* 2011;**51**:17–46.
- Wakita H, Nakamura Y, Kita I *et al.* Hydrogen release: New indicator of fault activity. *Science (80- )* 1980;**210**:188–90.
- Wang J, Wan W. Factors influencing fermentative hydrogen production: a review. *Int J Hydrogen Energy* 2009;**34**:799–811.
- Welch SA, Taunton AE, Banfield JF. Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiol J* 2002;**19**:343–67.
- Wu X, Holmfeldt K, Hubalek V *et al.* Microbial metagenomes from three aquifers in the Fennoscandian shield terrestrial deep biosphere reveal metabolic partitioning among populations. *ISME J* 2016:1192–203.
- Zerkle AL, Mikhail S. The geobiological nitrogen cycle: From microbes to the mantle. *Geobiology* 2017, DOI: 10.1111/gbi.12228.