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# Corrosion of Copper in Anoxic Ground Water in the Presence of SRB

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Copper is used in various applications in environments favoring and enabling formation of biofilms by naturally occurring microbes. Copper is also the chosen corrosion barrier for nuclear waste in Finland. The copper canisters should have lifetimes of 100,000 years. Copper is commonly considered to be resistant to corrosion in oxygen-free water. This is an important argument for using copper as a corrosion protection in the planned canisters for spent nuclear-fuel encapsulation. However, microbial biofilm formation on metal surfaces can increase corrosion in various conditions and provide conditions where corrosion would not otherwise occur. Microbes can alter *p*H and redox potential, excrete corrosion-inducing metabolites, directly or indirectly reduce or oxidize the corrosion products, and form biofilms that create corrosive microenvironments. Microbial metabolites are known to initiate, facilitate, or accelerate general or localized corrosion, galvanic corrosion, and intergranular corrosion, as well as enable stress-corrosion cracking. Sulfate-reducing bacteria (SRB) are present in the repository environment. Sulfide is known to be a corrosive agent for copper. Here we show results from corrosion of copper in anoxic simulated ground water in the presence of SRB enriched from the planned disposal site.

***Keywords:*** *Copper, Repository, Sulfate reducing bacteria, Anoxic ground water, Corrosion*

## 1. Introduction

Copper is used in various applications to inhibit microbial biofilm formation in environmental conditions that otherwise would favor the formation of biofilms. Copper is also the corrosion barrier of choice for the disposal canister in the Finnish nuclear waste disposal program, because under oxygen free conditions copper is assumed to be resistant to corrosion. The disposal of high-level radioactive waste is based on the usage of multiple barriers. These barriers are the form of used nuclear fuel, the disposal canister, bentonite buffer, backfill materials in the tunnels and lastly the surrounding bedrock. The disposal canister plays a major role of these barriers. The copper canisters should have lifetimes of 100,000 years to prevent the release of radioactive nuclides to the surrounding environment.

The failure mechanisms of copper canister have been considered by means of models developed based on literature, of corrosion tests simulating the disposal conditions and of copper items found in nature [1]. However, there are only few studies done in the presence of microbes. The activity of microbes attached to surfaces and the properties of formed biofilms are essential factors when considering the possibility of microbiologically influenced corrosion (MIC) [2-4]. Under the biofilm the conditions may differ remarkably from the surrounding solution and thus induce circumstances where the corrosion is locally increased. In addition to the microbes attached to the surface of copper, also the microbiological activity in bentonite or in its vicinity producing the corrosive ingredients for copper, like acetate, nitrogen compounds or sulfides, can change the conditions and thus substantially accelerate the corrosion. These corrosive products may migrate to the vicinity of the copper canister especially when the bentonite buffer is unevenly swelled or compacted.

The microbiological surveys from the disposal site have shown that there are several active groups of microorganisms in deep bedrock ground water, which might have an effect to the different corrosion mechanisms of copper. Sulfate reducing bacteria (SRB) producing sulfide, which is a known to be corrosive to copper, are an example of these microorganisms. Besides SRB also betaproteobacteria are detected from deep bedrock ground water. Betaproteobacteria have been seen in biofilms under which copper plumbing pipes had notable corrosion failures [5]. Studies at the nuclear waste repository site have also shown that the microorganisms natively present have

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great affinity to attach to surfaces and form biofilm [6]. Jägevall *et al*. [7] demonstrated that the composition of the bacterial community forming biofilm on rock surfaces under in situ conditions differed greatly from that of the planktonic bacteria in the ground water.

Our aims in this study were to estimate and clarify the effects of microbiological activity on the corrosion behavior of copper canister in the nuclear waste disposal conditions in Finland. This part of the study is concentrated on the anaerobic state of the disposal period and the effect of SRB in a simulated ground water environment.

## 2. Experimental Procedure

The laboratory experiment was designed to simulate the final stage of the deep geological nuclear waste repository, when the temperature has already stabilized to the level of the surrounding bedrock and where all oxygen introduced at the construction stage has been consumed. Copper samples were in direct contact with the ground water, simulating the scenario where bentonite buffer has lost its full performance. The chemistry of artificial ground water was calculated to simulate the ground water of the repository, which had been stabilized with bentonite. At this stage the most important cause of microbially induced corrosion is thought to be SRB. Corrosion was studied with on-line electrochemical methods as well as with gravimetric measurements. Molecular biological methods were applied to study the microbial community.

**Table 1 The composition of simulated ground water**

| Compound | mg $L^{-1}$ |
|---|---|
| K | 54.7 |
| Ca | 280.0 |
| Cl | 5274.0 |
| Na | 3180.2 |
| $SO_4$ | 595.0 |
| Br | 42.3 |
| $HCO_3$ | 13.7 |
| Mg | 100.0 |
| Sr | 8.8 |
| Si | 3.1 |
| B | 1.1 |
| F | 0.8 |
| Mn | 0.2 |

The material used in this study was oxygen-free phosphorus- doped (OFP) copper, the same material that will be used as the outer shell material of the nuclear waste disposal canisters in Finland. Copper coupons for gravimetric measurements (3 parallel) and for microbiological (5 parallel) studies (size 70 mm × 25 mm × 3 mm) as well as those for electrochemical measurements (3 pieces, exposed surface 1 $cm^2$) were ground to 600 grit. Prior to the experiments the length, width and thickness of the coupons were measured. The surfaces were cleaned with deionized water and ethanol and air-dried prior to sterilizing in 70 % ethanol. Coupons for weight loss measurements were weighed with the accuracy of 0.1 mg.

Anoxic simulated ground water was prepared (Table 1) and sterilized by autoclaving (121 °C, 20 min, 1 atm over pressure). The pH of the simulated ground water was set to 7.9. Sodium lactate ($C_3H_5NaO_3$, 1 mg $L^{-1}$) was added as carbon and energy source for the bacteria before sterilizing. The oxygen level of the simulated ground water was measured with Hach Sension 156 meter before and after the experiment. The oxygen level was 0.45 mg $L^{-1}$ at the beginning of the experiment.

The experiments were performed in gas tight glass mesocosms (12 L), which had been acid washed and rinsed both with 70% ethanol and sterile MQ-water before the experiments started. Copper coupons were aseptically transferred to the mesocosms and the mesocosms were flushed with nitrogen gas before and during the transfer of oxygen free simulated ground water. For the biotic environment an enrichment of SRB was prepared from ground water retrieved from a deep borehole (417.5 m) of the planned disposal site. The enrichment was grown on *Desulfovibrio* medium 63 broth (DSMZ) at the temperature of 14 °C corresponding to the temperature at the planned disposal site. The enrichment culture was renewed monthly before the inoculation into the mesocosms. Mesocosms representing a sterile environment containing the simulated ground water but without microbe additions was used as a control environment. The temperature of the experiments was 10 °C ± 2 °C and the duration was 4 months. To ensure anaerobic conditions, the mesocosms were placed in airtight plastic barrels that were flushed regularly with nitrogen gas.

### 2.1 Electrochemical measurements and weight loss evaluations

During the exposure of copper to the simulated ground water the open circuit potential of one of the electrochemical specimens was measured continuously. A designed Ag/AgCl reference electrode filled with 0.15 M KCl was used with potential measurements. All the results

here are converted and shown in standard hydrogen electrode (SHE) scale.

Linear polarization resistance (LPR) measurements were used for determining the general corrosion rate. In the LPR experiment the current was recorded while voltage was swept over a small range of potential close to Eoc (± 20 mV, 10 mV/min). A polarization resistance is inversely proportional to the corrosion rate:

$$Rp = B/icorr,$$
$$\text{where } B = (\beta a\ \beta c)/(2.303 \times (\beta a + \beta c)) \quad (1)$$

The Beta coefficient values were obtained experimentally from Tafel measurements. Tafel measurements, Eoc ± 30 mV, 10 mV/min, were performed prior to each linear polarization resistance measurement. All these measurements were performed with a Reference 600TM potentiostat (Gamry Instruments) using DC105 DC Corrosion software. The counter electrode was platinum and another test specimen was used as a reference electrode.

After the exposure (4 months) in biotic and sterile simulated ground water the copper coupons (3 parallel) were removed under nitrogen flow from the mesocosms, immersed quickly in ethanol, air-dried and stored in a desiccator. At the end of the exposure pH, conductivity and oxygen content of both the biotic and sterile glass mesocosms were measured. For the gravimetric analysis the coupons were weighed, cleaned with brush and chemically. The cleaning solution was as follows: 500 ml $H_2O$ + 500 ml HCl + 3.5g hexamethyltetra amine. The chemical cleaning procedure was performed 4 times (5 min). To determine the mass loss of the base metal when removing the corrosion products, a replicate uncorroded control coupon was cleaned by the same procedure as that being used on the test coupons. The mass losses were determined and from the mass losses the average corrosion rates (μm $a^{-1}$) were calculated.

All corrosion coupons were inspected visually and under a stereomicroscope before and after the removal of corrosion products. Each metal coupon for corrosion studies was imaged using a digital camera. The corrosion products of selected coupons were analyzed with an Energy-Dispersive X-ray Spectrometer (EDS) coupled to a Scanning Electron Microscope (SEM). The mineral composition of the corrosion products was analyzed with X-Ray Diffractometry (XRD).

### 2.2 Microbiological studies

The copper coupons (3 parallel) for microbiological studies were removed from each mesocosm aseptically under nitrogen flow and subsequently stored on dry ice in sterile plastic tubes until DNA extraction. The biomass from two parallel water samples of 250 mL were collected on sterile 0.22 μm -filtration units from each mesocosm for subsequent DNA extraction. The biofilm was extracted from the surface of the copper coupons by bead beating the coupons in 10 mL sterile phosphate buffered saline (PBS) and Tween®20 (1 μL Tween®20 1 $mL^{-1}$ PBS) for 20 minutes at 150 rpm agitation and followed by ultra-sonication for 3 min. The biomass released from the copper coupons was subsequently collected on 0.22 μm -filtration units for subsequent DNA extraction.

The filtration units were prepared for DNA extraction by first breaking the filter units with sterile pliers and cutting out the filter membranes. The DNA was subsequently extracted using the PowerWater® DNA Isolation kit (MoBio Laboratories, Inc., CA, USA) in accordance with the manufacturer's protocol and the DNA was eluted in 100 μL elution buffer supplied by the manufacturer. Negative DNA extraction controls were included in the DNA extractions.

As a proxy for bacterial biomass, quantitative PCR (qPCR) was used to determine the amount of 16S rRNA gene copies in the water and on the surface of copper. qPCR was performed in 10 μL reaction volumes using the LightCycler® 480 qPCR machine and LightCycler® 480 Software 1.5.0 (Roche Applied Science, Germany). The reaction mixture contained 1 μL template, standard dilution or water, 1 × KAPA SYBR® FAST Universal qPCR Master Mix (KAPA Biosystems, MA, USA), 2.5 μM of both forward and reverse primer (P1 and P2, [8]) and nuclease free water. A ten-fold dilution series of plasmids containing the bacterial 16S rRNA gene ranging from $10^1$ to $10^9$ copies per reaction was used to estimate the concentration of 16S rRNA gene copies in the samples as well as no template controls. The PCR program consisted of an initial 15 min incubation at 95 °C, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 35 s and extension 72 °C for 30 s, and with final extension at 72 °C for 3 min. Sample fluorescence was measured at the end of each elongation phase. Subsequently, a melting curve was recorded, to test the specificity of the qPCR, with a program consisting of 10 s of denaturation at 95 °C, 1 min of annealing at 65 °C, and a melting and continuous measuring step rising gradually (20 °C $s^{-1}$) to 95 °C.

## 3. Results and Discussion

The continuously monitored open circuit potentials of copper in both environments (biotic and sterile) are shown in Fig. 1. The corrosion rates determined from the linear

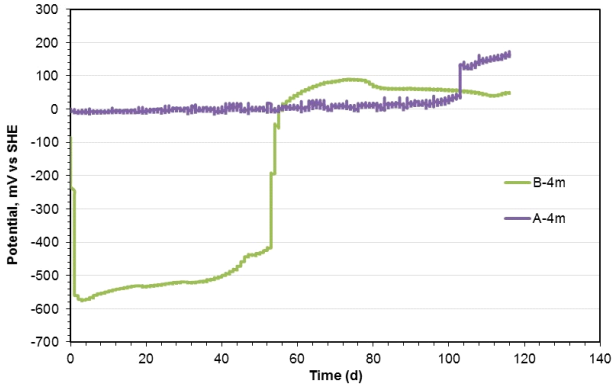



**Fig. 1 Open circuit potentials of copper in biotic (B-4m) and sterile (A-4m) anoxic simulated ground water.**

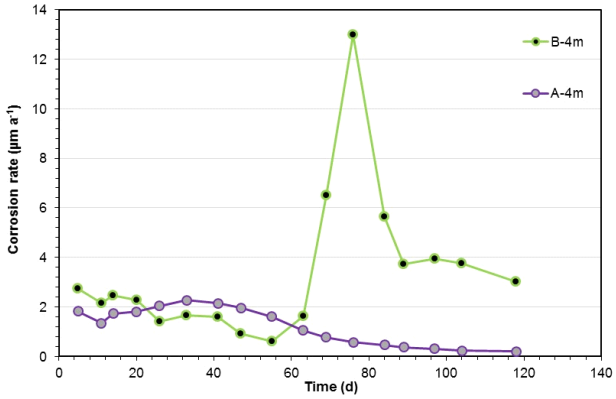



**Fig. 2 Corrosion rate of copper calculated from the linear polarization resistance measurements in biotic (B-4m) and sterile (A-4m) anoxic simulated ground water.**

polarization resistance (LPR) measurements are shown in Fig. 2.

The inoculation of microbes caused immediately a significant decrease (to -0.57 V vs SHE) in the open circuit potential compared to the sterile environment (-0.01V), Fig. 1. However, soon after the drop the potential started to increase first slowly, but after 55 days of immersion a more rapid increase was seen and the potential reached and exceeded the level of potential of the copper in the sterile environment. This increase is supposed to be due to biotic formation of sulfide that has reacted with copper forming a copper sulfide layer on the surface of the copper coupon. The open circuit potential of the copper in the sterile environment remained rather stable during almost the whole immersion period, the variation being from -0.01 to 0.025 V until the last couple of weeks when the potential increased to the level of 0.16 V.

In the beginning of the exposure the corrosion rates were around 2 µm $a^{-1}$ in both environments, Fig. 2. The corrosion rate in the biotic environment was first a little higher than in the sterile environment until the corrosion rate in the biotic environment started to increase rapidly to 8 µm $a^{-1}$. This rapid increase occurred simultaneously with the sudden increase of the copper potential in the same environment, Fig. 1. The smaller increase of copper potential in the sterile environment did not show similar effects in the corrosion rate. However, the last LPR measurement was done only a day after the potential had started to increase.

The pH values had decreased in both environments during the exposure to 7.2 in the biotic and 6.9 in the sterile environment from the original 7.9. The conductivity was the same in both environments, 17 mS $cm^{-1}$ and the oxygen content was 0.3 mg $L^{-1}$ in the biotic and 1.0 mg $L^{-1}$ in the sterile environment. However, these values are probably higher than actual values in the repository environment since reliable measurement requires flowing conditions, which was not possible to simulate in this experimental setup.

In the simulated ground water containing SRB the copper coupons had turned almost black and had a quite even but rather thin deposit layer, Fig. 3. In contrast, in the sterile simulated ground water the copper coupons had a dark red, thin layer on the surfaces, Fig. 4. One coupon of each group was chosen for the EDS-analyses. The deposit layer on the biotic coupons had copper (Cu), sulfur (S), oxygen (O) and carbon (C) as the main components and smaller amounts of chlorine (Cl), phosphorus (P), calcium (Ca) and aluminum (Al). The amount of sulfur (S) varied from 5 w-% to 13 w-% in weight loss coupons and in the electrochemical specimen even higher amounts (19 w-%) were locally detected. Copper (Cu) and oxygen (O) were the main components on the deposits of the coupons immersed in sterile simulated ground water. In these coupons some areas with darker deposits contained a high amount of carbon (C) and nitrogen (N). In some areas also silicon was quite high. Chlorine (Cl), aluminum (Al) as well as occasionally small amounts of sulfur (S) (≤ 1 w-%) were detected.

The deposits on the electrochemical specimens were analyzed with XRD to reveal the mineral composition of the layers. The highest reflections were caused by metallic copper in both of the specimens. Besides copper, also chalcocite ($Cu_2S$) and possibly small amounts of cuprite ($Cu_2O$) and tenorite (CuO) were detected in the specimen from the biotic environment. In the specimen from the sterile environment only cuprite could be detected in addition to metallic copper. This corresponded well with the open circuit potentials set up to the potential/pH equilibrium diagram (Pourbaix diagram) of copper sulfur system. Potential/pH diagrams show possible stable phas-


**Fig. 3 The copper coupons of the biotic mesocosms for weight loss measurements (at the left) and those for electrochemical measurements (at right) after 4 months of immersion in anoxic simulated ground water with SRB. Above coupons after exposure and below the chemically cleaned coupons.**

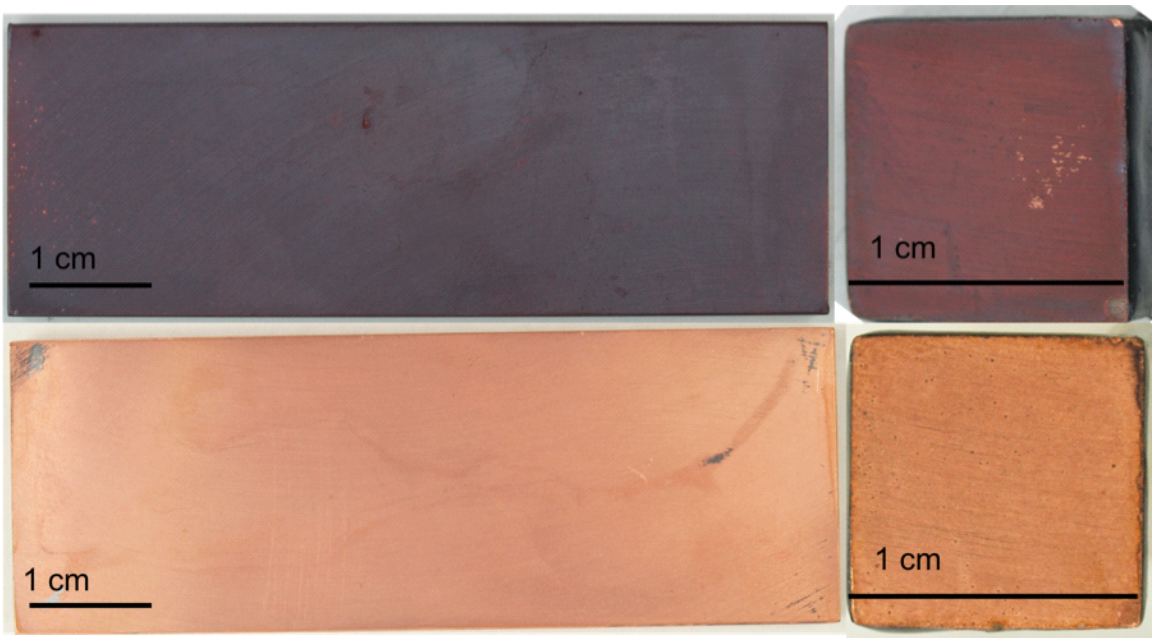



**Fig. 4 The copper coupons of the sterile mesocosms for weight loss measurements (at the left) and those for electrochemical measurements (at right) after 4 months of immersion in anoxic sterile simulated ground water. Above coupons after exposure and below the chemically cleaned coupons.**

es of an aqueous electrochemical system. The open circuit potential of the copper in the biotic environment with SRB was in the beginning of the exposure at the stability domain of copper (I) sulfide, chalcocite and increased later to the stability domain of copper (I) oxide, cuprite. In the sterile simulated ground water the open circuit potential remained at the stability domain of cuprite throughout the immersion time.

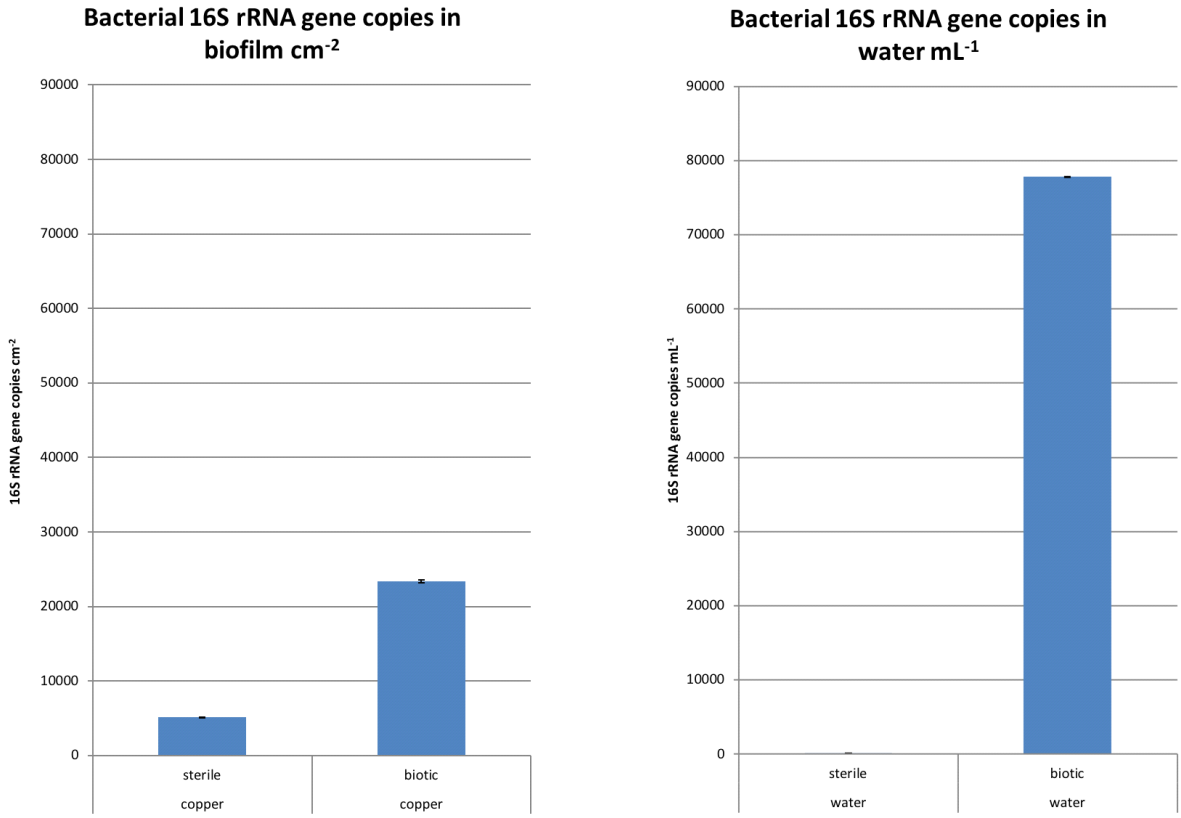
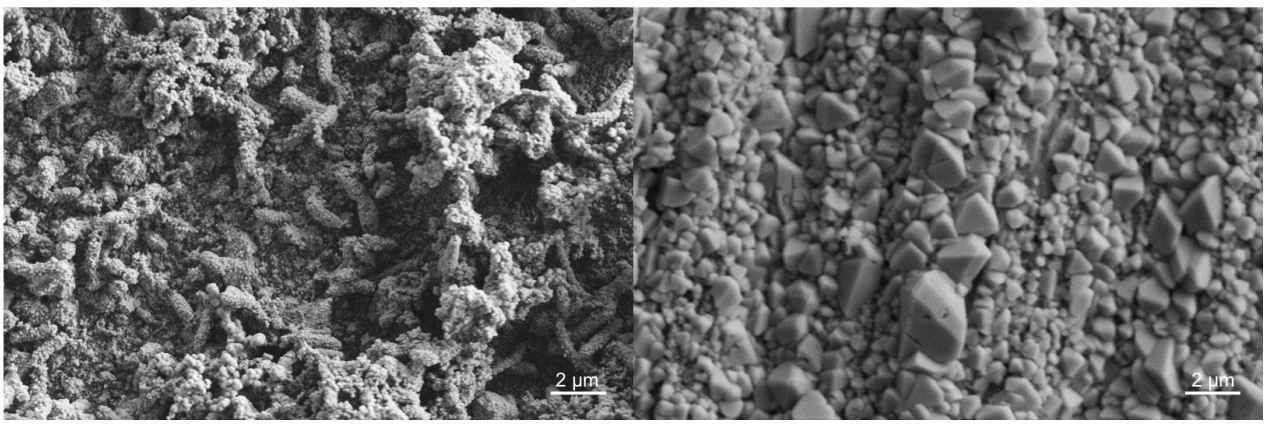



**Fig. 5 The amount of bacterial 16S gene copies detected by qPCR area ($cm^2$) of the copper coupons (left) and per volume (mL) of the simulated ground water (right).**




(a)

**Fig. 6 (a) SEM image of the surface of copper in the biotic environment.**

(b)

**Fig. 6 (b) SEM image of the surface of copper in the sterile environment.**

The gravimetric results confirmed a higher corrosion rate of copper in the presence of SRB in the simulated ground water. The corrosion rate calculated from the weight loss measurements was 6.5 µm $a^{-1}$ with SRB compared to 2.1 µm $a^{-1}$ in the sterile simulated ground water. After the pickling procedures the coupons were examined under a low magnification microscope to reveal the nature of the corrosion failure. Most of the surface of the copper coupons immersed in the biotic environment had corroded generally exposing the grain boundaries. However, there

were also areas where more localized corrosion could be seen. The surface of the coupons immersed in the sterile simulated ground water was more even and corrosion was concentrated in smaller areas where also some shallow pits could be seen.

The qPCR analyses showed that the inoculation of bacteria to the simulated environment was successful and that the bacteria were both planktonic freely in the water and attached to the surface of copper forming biofilm (Fig. 5). The amount of bacterial 16S rRNA gene copies in the biofilm on the surface of copper was $2.3 \times 10^4$ gene copies $cm^{-2}$ (Fig 5). The amount of bacterial 16S rRNA gene copies in the simulated ground water was $7.7 \times 10^4$ $mL^{-1}$ (Fig. 5). The amount of 16S rRNA genes detected from the sterile environment was low showing that changes in this environment were not caused by microbial functions. The SEM imagining also supported these findings, showing microbes on the surface of copper in the biotic experiment but not in the sterile one (Fig. 6a and b).

## 4. Summary

The corrosion behavior of copper was studied in simulated anoxic ground water in the presence of sulfate reducing bacteria (SRB) or in sterile reference environment. The results of these preliminary studies show that the sulfate reducing bacteria enriched from native groundwater at the repository site clearly accelerate the corrosion of copper and have the ability to form dense biofilm on the surface of copper. Corrosion inducing ability of SRB could be seen by electrochemical measurements (LPR) and the gravimetric results confirmed the results. In the environment with SRB copper(I) sulfide deposit was detected under which general corrosion could be detected. Increase detected in OCP values in SRB environment is due to formation of biotically produced copper(I) sulfide layer on surface of copper. In addition to general corrosion, some areas with more localized corrosion were detected. In addition, in presence of SRB the grain boundaries were opened slightly. In the sterile environment the deposit developed during the exposure was copper(I) oxide and a light general corrosion with some shallow pits were detected only on smaller areas of the surface. The results presented here demonstrated that ground water microorganisms affect the deposit formation on surface of copper and also induce corrosion.

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