Real-Time Electrochemical Measurements Of Carbon Steel In Ground Water With Sulfate Reducing Bacteria Enrichment – 15604

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ABSTRACT

A new laboratory system for studying the microbiological corrosion of decommissioning waste was designed and developed during this project. The material in this part of the laboratory studies was carbon steel (AISI/SAE 1005/UNS G10050). In order to estimate the corrosive effects of microbial activity both biotic and abiotic (containing the biocides methylisothiazolinone and glutaraldehyde) mesocosms were prepared. To enhance the effects of sulfate reducing bacteria an enrichment of these bacteria isolated from the native drill hole water from the repository site was inoculated in to the biotic mesocosms. Microbiological corrosion was studied using new electrochemical technologies, such as multi-electrode arrays sensors, and molecular biology methods in the laboratory.

INTRODUCTION

Low and intermediate level radioactive waste is generated during the operation and decommissioning of nuclear power plants. In Olkiluoto, Finland, Teollisuuden Voima Oyj (TVO) has been disposing operational waste into an underground final repository since 1992. The silos excavated into the bedrock reach the level of 100 m below sea surface. This waste contains pipes, valves, filters etc., with the metallic components being primarily made of carbon and stainless steel. A durability of hundreds years is required for a repository system containing low and intermediate waste.

The ground water at this depth is anoxic and the concrete used in the construction of the silos or as packing material of waste generates an alkaline environment. In oxygen-free alkaline water, the corrosion rate of carbon steel is typically low, unless microbial activity exists in the surrounding environment. The pH starts to decrease over time, for example due to the carbonization of concrete. As a consequence of the decrease in pH and the following accelerated corrosion of the metallic waste by microorganisms, radioactive nuclides may be released into groundwater and transferred to neighboring areas of the repository. It is well known that the groundwater at the repository depth contains considerable microbial population, up to 10^5 microbes mL^{-1} [1]. These microorganisms may significantly contribute to the corrosion of carbon steel. Microorganisms are able to accelerate several types of steel corrosion, such as general corrosion and localized corrosion. Localized corrosion includes pitting and stress cracking corrosion. It has been demonstrated that the average corrosion rate may be as high as 63 µm a^{-1} and localized corrosion rates even higher in the repository environment [2]. Such high corrosion rates in anoxic groundwater are not possible without microbial acceleration of the corrosion process. Especially biofilm formed on the surface of carbon steel by microorganisms is known to accelerate the corrosion.

In nutrient poor conditions, such as deep anoxic groundwater, certain sulfate reducing bacteria and methanogenic archaea may use metallic iron (Fe^0) as their sole source of electrons [3]. By using the metallic iron as electron donor microbes can accelerate the corrosion reaction even more aggressively than by producing aggressive agents, such as organic acids or sulfide, or by consuming the hydrogen, which are the other suggested reactions for how microorganisms are thought to accelerate corrosion of metals.

The effects of microbial activity on the corrosion of low and intermediate level waste have to be thoroughly studied and understood in order to ensure the safety during the whole repository time scale.

Here we studied by real-time electrochemical measurements how the sulfate reducing bacteria enriched from native groundwater will accelerate the corrosion of carbon steel.

METHODS

Experimental setup

A new laboratory mesocosms for investigating the microbiologically induced corrosion (MIC) of decommissioning waste metals was designed and developed. Experiments were performed in 12 L glass mesocosms where the materials under the investigation were inserted. Groundwater (pH 7.7, O_2 0.21 mg L^{-1}, γ 2.3 mS cm^{-1}, redox potential -7 mV vs SHE, alkalinity 5.0 mmol L^{-1}, Cl^- 567 mg L^{-1}, SO_4^{2-} 137 mg L^{-1}, TOC 9 mg L^{-1}) for the experiment was lead directly from the drill hole at the disposal site to the N_2 flushed glass vessels. The conductivity of the ground water was measured with a conductivity meter, redox potential, oxygen level and pH with a combination-meter (HACH Sension 156) immediately on site and at the end of the incubation period. The chemical composition of the groundwater was analyzed in the beginning and end of the experiment. In order to estimate the corrosive effects of microbial activity both biotic and abiotic mesocosms were prepared. Before starting the measurements, the microbial activity in the abiotic reference mesocosms was reduced by adding the biocides methylisothiazolinone (6 mL 9.5% v/v, ProClin 950, Sigma-Aldrich) and glutaraldehyde (3.6 mL 25% v/v, VWR International), anaerobically to the mesocosms using sterile plastic syringes. To accelerate the microbially induced corrosion an inoculant (70 mL) containing sulfate reducing bacteria (SRB) enriched from the drill hole water was added to biotic mesocosm. SRB from the groundwater were anaerobically enriched using the *Desulfovibrio* medium 63 broth (DSMZ) at the temperature of 14°C. The enrichment culture was recultivated monthly before it was inoculated in to the biotic mesocosms. The mesocosms were sealed with ground glass caps and the joints were sealed with silicon grease and tightened with steel wire in order to prevent oxygen from entering the mesocosms. The immersion time was one year and the exposure was performed at the temperature of 10 ± 2 °C.

Materials

Low alloy carbon steel (AISI/SAE 1005/UNS G10050) from cold-rolled thin sheet was used as materials for test coupons, Table 1. The coupon size for electrochemical studies was 10 mm × 10 mm (exposed area 1 cm^2) and the surfaces were ground to 600 grit. For microbiological studies as well as for gravimetric studies (will be reported separately) the size of the coupons was 71 × 26 × 1 mm and the surfaces as received. All the steel coupons were cleaned with deionized water and ethanol and air-dried and stored in a desiccator until the start of the test. Prior to the incubation the specimens were sterilized in 70% ethanol.

TABLE 1. The chemical composition of carbon steel, wt.%

C	Si	Mn	S	P	Cr	Ni	Mo	Cu	Al	W	V	Ti	Co	B
0.03	0.030	0.22	0.005	0.006	0.02	0.04	0.005	<0.003	0.054	<0.01	<0.003	0.001	0.01	0.0013

Electrochemical measurements

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 open circuit potential (Eoc ± 20 mV, 10 mV/min). A polarization resistance is inversely proportional to the corrosion rate (icorr):

$$Rp = B/icorr, \text{ where } B = (\beta a\ \beta c)/(2{,}303 \times (\beta a + \beta c)) \qquad (Eq.1)$$

The Beta coefficient values were obtained from Tafel Plots. All these measurements were performed with 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. Besides general corrosion also measurements for localized corrosion were performed with multielectrode array sensors (MASS, NanoCorr A-50 Coupled Multielectrode Analyzer, Corr Instruments LLC) of carbon steel (type 1008)). Each probe had 9 electrodes flush-mounted in epoxy.

During the exposure the potential of one of the tree electrochemical specimens was followed continuously. The reference electrode for the potential measurements was a Mg-electrode. In the figure 1 the potentials here are shown in SCE (saturated calomel electrode) scale. The difference between standard hydrogen electrode (SHE) and SCE at the temperature 10 °C is approximately 0.251 V.

Microbiological analyses

To analyze the planktonic microbial community, the microbial biomass of 500 mL subsamples was collected anaerobically from water in the mesocosms on the 0.22 µm CA-filters (Corning) at the end of experiment and the samples were stored at -80°C until DNA extraction. The biofilm was extracted from the surface of the steel coupons by bead beating coupons in 10 mL sterile phosphate buffered saline (PBS) and Tween®20 (Merck, Germany) (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 steel coupons was subsequently collected on 0.22 µm Sterivex™ -filtration units (Millipore, MA, USA) for subsequent DNA extraction. The filters of the water samples and biofilm were prepared for DNA extraction by first breaking the filter units with sterile pliers and cutting out the filter membranes using sterile scalpels. 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 50 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 each sample. The amount of sulfate reducing microbes was determined based on the number of copies of the gene for the β-subunit of the dissimilatory sulfite reductase (*dsr*B). The presence of methanogenic archaea was studied using qPCR targeting the gene for the α-subunit of the methyl coenzyme-M reductase (*mcr*A). 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 DNA 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 for 16S rRNA gene, DSRp2060F and DSR4R for *dsr*B gene, ME1 and ME3 for *mcr*A gene) and nuclease free water [4, 5, 6, 7]. A ten-fold dilution series of plasmids containing the bacterial 16S rRNA gene, *dsr*B gene or *mcr*A gene ranging from 10^1 to 10^9 copies per reaction was used to estimate the concentration of the corresponding gene copies in the samples and 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.

RESULTS AND DISCUSSION

The open circuit potentials of the carbon steel were low already in the beginning of the measurement period (Figure 1). The potential in the abiotic environment has been more stable (between -700 and -800 mV vs SCE corresponding - 449 mV to – 559 mV vs SHE) and most of the time on a slightly higher level than that of the specimen in the biotic environment. The potential of carbon steel in the biotic SRB enriched environment fluctuated more, the lowest values being -932 mV (-681 mV vs SHE) and the highest -726 mV (-475 mV vs SHE). These values fall close to the stability domain of iron sulfide in the potential/pH equilibrium diagram (Pourbaix diagram) of an iron sulfur system. Potential/pH diagrams show possible stable phases of an aqueous electrochemical system.

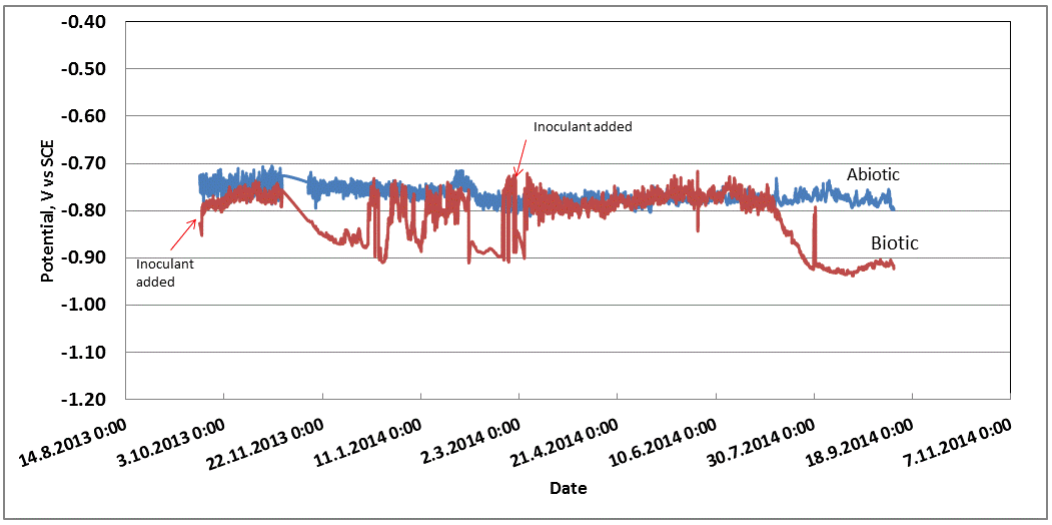

Fig. 1. Open circuit potentials of carbon steel in low oxygen ground water.

According to the LPR measurements the general corrosion rate of carbon steel both in enriched biotic and abiotic environment was low (from 2 to 16 µm per year) during the first two months of immersion (Figure 2). After that the corrosion rate in the biotic environment started to increase whereas that of the specimen in the abiotic environment decreased and remained at the lower level until started to increase in the middle of immersion time and stayed almost at that same level (20 µm a^{-1}) to the rest of the exposure time. The reason of this sudden increase of corrosion rate in abiotic environment is not clear. It is known however, that the used biosides cause a certain amount of corrosion according to the weight loss measurements (results not shown here). The corrosion rate of specimen in the biotic SRB enriched environment increased to 35 µm a^{-1} and whereafter it started to decrease to the level it had been before the rise.

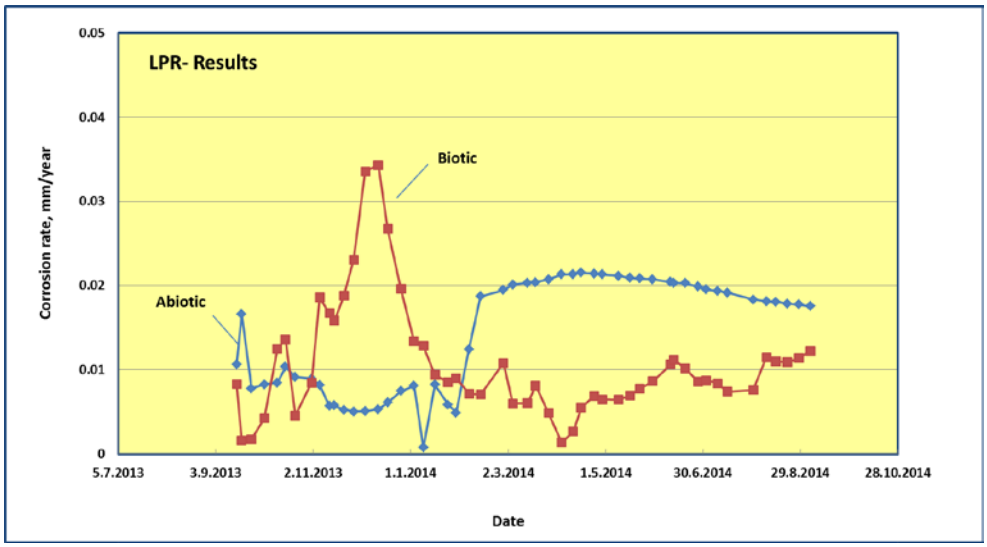

Fig. 2. Corrosion rate of carbon steel in low oxygen water determined by LPR measurements.

The inoculation did not show immediately increase in the general corrosion rate, but it took almost two months before any differences between the abiotic and biotic environment could be seen. The second addition of enriched SRB solution showed only a very minor increase in the corrosion rate just after the amendment (in the beginning of March) but after that soon first lowered to 1 µm a^{-1} and then started to gradually increase again.

Without SRB enrichment in the same groundwater environment the general corrosion rate determined by LPR measurements showed lower values; e.g. less than 20 µm a^{-1} [8]. Whereas the addition of nutrients (methane or glucose) increased the corrosion rate of carbon steel much more, with methane the highest measured rate was almost 500 µm a^{-1} and with glucose more than 200 µm a^{-1} [8]. However, it has to be taken into account that these were the highest values during the measuring period and that the real cumulated corrosion rate is much lower, as was shown with gravimetric results in the earlier study [8]. The gravimetric results of this study are not discussed here.

The localized corrosion rate (average of all 9 electrodes, multielectrode array sensor) of carbon steel in biotic environment enriched with SRB was clearly higher than that in abiotic environment, but again in the beginning of immersion period the localized corrosion rate fluctuated and the difference between abiotic and biotic environment was not so clear (Figure 3). After the second addition of inoculant it took almost three months before the distinct rise in the localized corrosion rate started in the enriched biotic environment. The difference between abiotic and biotic environments becomes more prominent when examining the highest localized corrosion rate (of one of the nine electrodes which had been corroded most, Figure 4), where the highest corrosion rate in groundwater that had received SRB enrichment was over 900 µm a^{-1} compared to 100 µm in abiotic groundwater. These values are much higher than detected in an earlier study without inoculant addition [8], where the highest localized corrosion rate was around 350 µm a^{-1}.

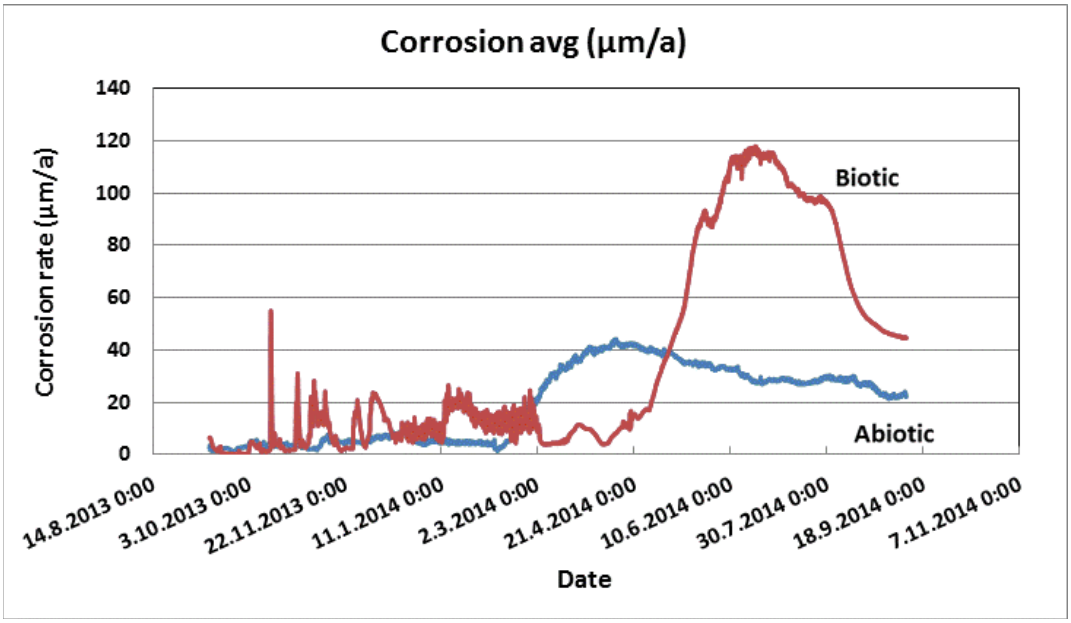

Fig. 3. Average localized corrosion rate of carbon steel in enriched biotic and abiotic ground water.

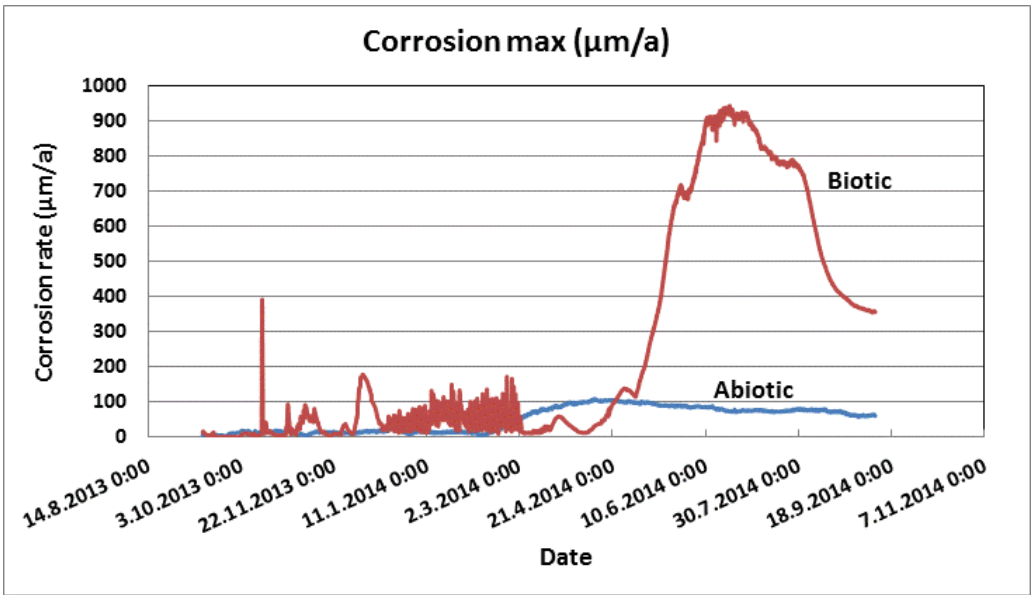

Fig. 4. The maximum value of localized corrosion of carbon steel in enriched biotic and abiotic ground water.

The concentration of bacterial 16S rRNA gene copies was analyzed using qPCR. The amount of 16S gene copies in ground water in the beginning of the experiment was 2.8×10^5 copies mL^{-1}. Biofilm formation on the surface of carbon steel was intense, showing 1.3×10^6 16S rRNA gene copies cm^{-1}, (Figure 5). The amount of SRB on the carbon steel surface was 4.7×10^3 copies cm^{-2} (Figure 6). The amount of metanogenic archaea on the surfaces of carbon steel was 3.3×10^3 copies cm^{-2}.

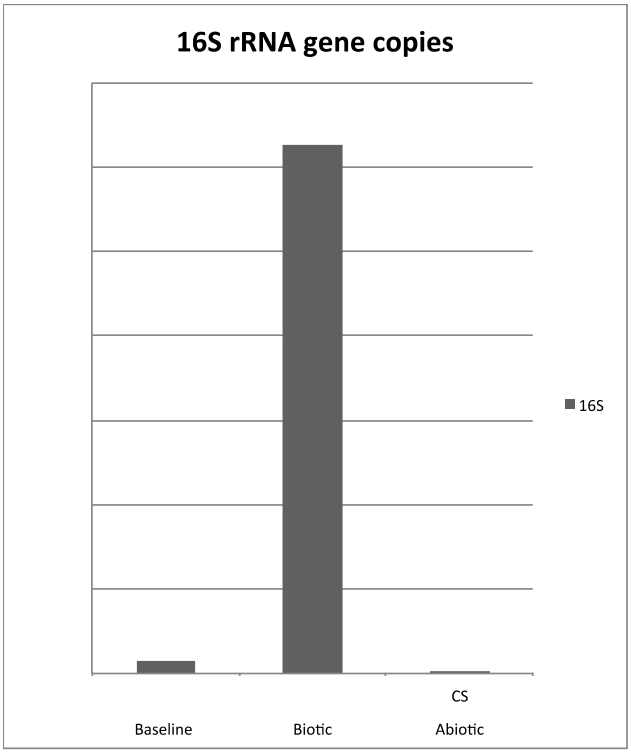

Fig.5. Number of 16S rRNA gene copies detected from the water at the beginning of the experiment (baseline) and biofilm formed on carbon steel (CS) surfaces. The concentration in water is given as gene copies mL^{-1} and for the biofilm as gene copies cm^{-2}

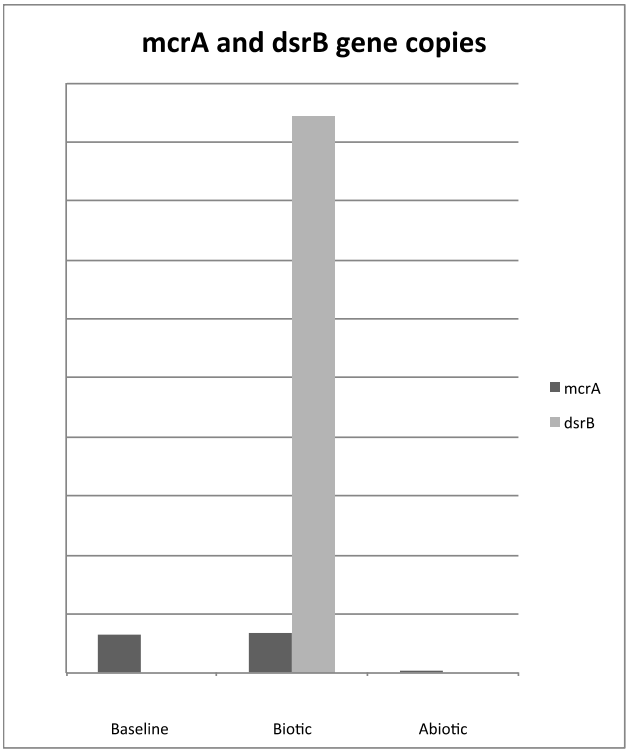

Fig.6. The number of *mcr*A and *dsr*B and gene copies detected from the water at the beginning of the experiment (baseline) and biofilm formed on carbon steel (CS) surfaces. The concentration in water is given as gene copies mL^{-1} and for the biofilm as gene copies cm^{-2}

The qPCR results show that the enrichment of SRB has been successful and intensive biofilm was formed on the surface of the carbon steel. The comparison of the electrochemical results with our earlier study shows that the microbial community interacting with the corrosion of carbon steel is probably much more heterogeneous than assumed here [8]. The corrosion induced by microbes that were enriched on *Desulfovibrio*-medium used here was milder than that of the native community induced by nutrient addition [8].

CONCLUSIONS

The results of these real-time electrochemical measurements showed that the addition of SRB enrichment to groundwater mesocosms caused higher corrosion rates both in the sense of general as well as localized corrosion compared to abiotic groundwater or natural groundwater without SRB enrichment. The general corrosion rate was however lower here than in the case of added nutrients.

However, the results obtained in this and previous studies show that the natural microorganisms inhabiting groundwater are able to form biofilm on carbon steel surfaces in different conditions. The microbial diversity in natural groundwater is vast and the microorganisms may be able to adapt to changing environmental conditions.

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