

# Trained to corrode: Cultivation in the presence of Fe(III) increases the electrochemical activity of iron reducing bacteria – An in situ electrochemical XANES study

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## ARTICLE INFO

### Keywords:

Microbiologically influenced corrosion

MIC

XANES

In situ electrochemical XANES

Iron reducing bacteria

*Shewanella putrefaciens* CN32

## ABSTRACT

This paper reports results from in situ electrochemical X-ray absorption near-edge spectroscopy (XANES) studies of the corrosion processes on model thin iron films in the presence of iron reducing bacteria *Shewanella putrefaciens*. Here we investigate the electrochemical activity of two cultures grown in the presence and absence of Fe(III) citrate in the culture medium. The XANES spectra and the OCP data of the Fe sample incubated with the culture grown in absence of Fe(III) did not show any significant changes during twenty hours of monitoring. In the case of the culture grown in Fe(III) containing medium, an accelerated dissolution of the iron film was observed together with the formation of a mixed Fe(II)-Fe(III) hydroxide surface layer. The open circuit potential (OCP) steadily approached the free corrosion potential of iron in neutral chloride containing electrolytes, indicating a continuous dissolution process without passivation.

## 1. Introduction

*Shewanella* species are electroactive bacteria capable of external electron transfer utilizing various metals as terminal electron acceptors [1–4]. For microbiologically influenced corrosion processes on stainless steels, electrochemical reduction of Fe(III) and Cr(VI) oxides and hydroxides by microorganisms can play an important role in the dissolution behavior and thus, the stability of the protecting passive film [5].

The analysis of the mechanisms of microbiologically influenced corrosion processes requires in situ tools combining spectroscopic and electrochemical methods. Ex situ investigation of corrosion products bares the risk of losing the original chemical state of surfaces and interfaces due to reoxidation reactions in contact with atmospheric oxygen during drying and sample transfer [6,7]. Moreover, in case of microbiologically influenced corrosion, the removal of the biofilm is necessary for the chemical analysis of the corrosion products at the metal surface by means of ultra-high vacuum based spectroscopic methods such as X-ray photoelectron spectroscopy (XPS). The available cleaning procedures again remove or alter the corrosion products and can lead to significant interpretation errors.

In their pioneering studies Davenport and Sansone, Kerkar et al.,

Virtanen et al. and Schmuki et al. have demonstrated the application of in situ electrochemical X-ray Absorption Near-Edge Spectroscopy (XANES) to the investigation of the changes in the passive film chemistry on iron [8–10] and iron/chromium alloys [10] as well as electrochemically induced changes in model iron oxide thin films [11]. Davenport and Sansone have proposed that the passive film formed on iron at high anodic potentials (+0.4 V vs. MSE) in a pH 8.4 borate buffer solution is composed of a composite of  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>/Fe<sub>3</sub>O<sub>4</sub> [8]. According to Kerkar et al. [10] the passive film on iron can be regarded as a disordered lepidocrocite ( $\gamma$ -FeOOH). In alkaline solutions the reduction processes result in the conversion of the Fe(III) containing passive film to a porous Fe(II) oxide/hydroxide layer, due to the lower solubility of Fe(II) at higher pH values [9]. Furthermore, in acidic solutions, it is reported that the dissolution of iron oxide films is strongly increased by the presence of Fe(II) in the oxide film [11]. In a recent study, Monnier et al. have investigated the reduction and reoxidation processes of iron corrosion products by means of transmission mode XANES. Their results indicate that the reduction of lepidocrocite and ferrihydrite yields magnetite (Fe<sub>3</sub>O<sub>4</sub>) and iron (II) hydroxides [6].

In this work, in situ electrochemical XANES was applied using model thin film substrates for the investigation of microbiologically influenced corrosion of iron in the presence of iron reducing *Shewanella*

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**Table 1**  
Composition of the *Shewanella* minimal medium.

NH <sub>4</sub> Cl	15.0 mM
Na <sub>2</sub> SO <sub>4</sub>	7.0 mM
MgSO <sub>4</sub>	0.4 mM
CaCl <sub>2</sub>	0.4 mM
Na glutamate	1.5 mM
KH <sub>2</sub> PO <sub>4</sub>	4.25 mM
Na <sub>2</sub> HPO <sub>4</sub>	4.25 mM
Na lactate	8.96 g L <sup>-1</sup>

*putrefaciens* cultivated in absence and abundance of Fe<sup>3+</sup> ions. The high concentration of Fe<sup>3+</sup> ions during cultivation aims at mimicking the conditions of the interface between the biofilm and solid substrate such as the ferric oxide rich passive film of stainless steels.

## 2. Experimental

*Shewanella putrefaciens* CN32 was grown in Erlenmeyer flasks at 28 °C under two different conditions to study the adaptation to iron metabolism. The regular CN32 culture was grown in a minimal medium with composition as indicated in Table 1 (*Shewanella* Minimal Medium (ShMM), adapted from M9 minimal medium, Miller et al., 1972 [12]). For the culture grown in the presence of Fe<sup>3+</sup>, referred to as FeCi culture, a mixture of 2-fold ShMM mixed 50:50 with 16 g/l Fe(III) citrate (Sigma Aldrich, pH 7) was used. For better comparability, both cultures were always inoculated and cultured overnight under identical conditions. The bacteria were washed three times with sterile ShMM via centrifugation at 1510 rcf for 4 min. Because of the high absorbance of iron citrate in the FeCi culture, the absorption at 600 nm (OD<sub>600</sub>) of the CN32 was taken as measurement reference. The final OD<sub>600</sub> was set to 0.1. For both systems, sterile control experiments were performed by extracting the supernatant via filtration of the cultures without any further dilution. These measurements are referred to as CN32-filtrate and FeCi-filtrate in the following sections.

XANES experiments have been performed at DELTA beamline 8 [13] using a Si(111) double-crystal monochromator and gas-filled ionization chambers as detectors for sample adjustment in transmission mode. Iron films of 80 nm thickness have been deposited by means of electron-beam evaporation deposition (10<sup>-8</sup> mbar, 15 W, CreaTec) on Kapton® foils. In the three-electrode cell assembly presented in Fig. 1,

the iron film is facing the electrolyte and serves as the working electrode. On the opposing side of the cell, a second Kapton® window allows transmission signals, when the cell is not filled. Due to a thickness of the electrolyte layer of 2 cm and the resulting strong parasitic absorption at energies around the Fe K-edge (7112.4 eV), in situ transmission mode XANES detection is not feasible. However, the optimized thickness of the iron film allowed an adequate iron signal in fluorescence detection with a large area PIPS (passivated implanted planar silicon) detector in close vicinity of the iron thin film sample. This fluorescence detector, positioned at 90° relative to the beam direction, was used to measure the intensity of the Fe-K $\alpha$  fluorescence radiation and obtain spectra as the ratio of the fluorescence detector signal to the signal of the incident photon beam from the first ionization chamber employing an integration time of 4 s/eV step.

For the in situ experiments, the assembled cell was placed in the beam path and position adjustment was performed based on the transmission signal. The cell was then filled with the respective medium and connected to a Gamry 600+ Potentiostat (C3 Prozessanalytik, Germany) to monitor the open circuit potential (OCP). A gold wire and a Ag/AgCl/3 M NaCl electrode (C3 Prozessanalytik, Germany) were used as counter and reference electrodes, respectively. The absorption spectra were collected within 60 eV below to 220 eV above the Fe K-edge. In the XANES region, equidistant energy steps of 0.3 eV were used. The time series were collected continuously overnight recording a full spectrum every hour. To assure that the observed affects are not stemming from beam induced damage, for the measurements with bacterial cultures, additional ex situ measurements with the same cell setup were performed at the beginning and the end of the incubation time. High purity reference samples of different iron oxides and hydroxides were made available at the beamline, either in form of pellets or dusted onto adhesive Kapton® tape [14,15]. The reference measurements were performed in transmission mode. The pre/post edge correction and edge calibration was performed using Athena (Demeter 0.9.26, [16]).

## 3. Results and discussion

The results of the in situ time series of XANES and OCP measurements on thin iron films during incubation with *Shewanella putrefaciens* CN32 cultivated in minimal media (CN32) and with addition of iron(III)

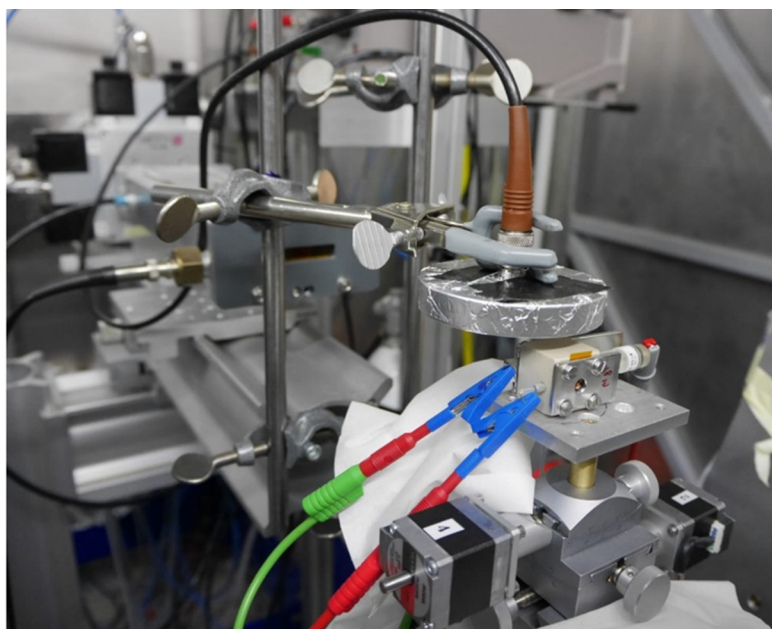
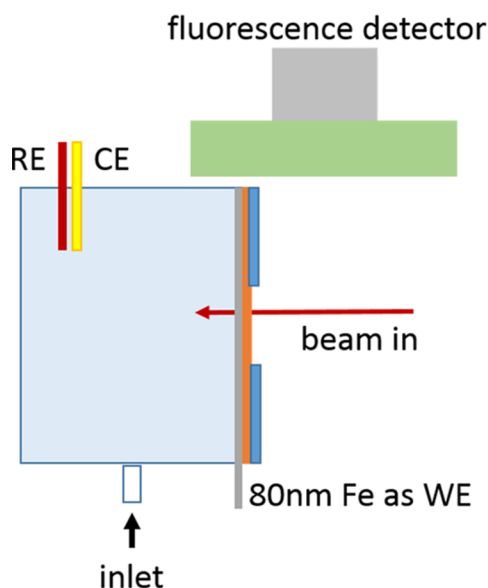


Fig. 1. (left) Schematics of the experiment setup and (right) installation at the beamline, cell volume: 0.555 ml, window 5.5 mm diameter.

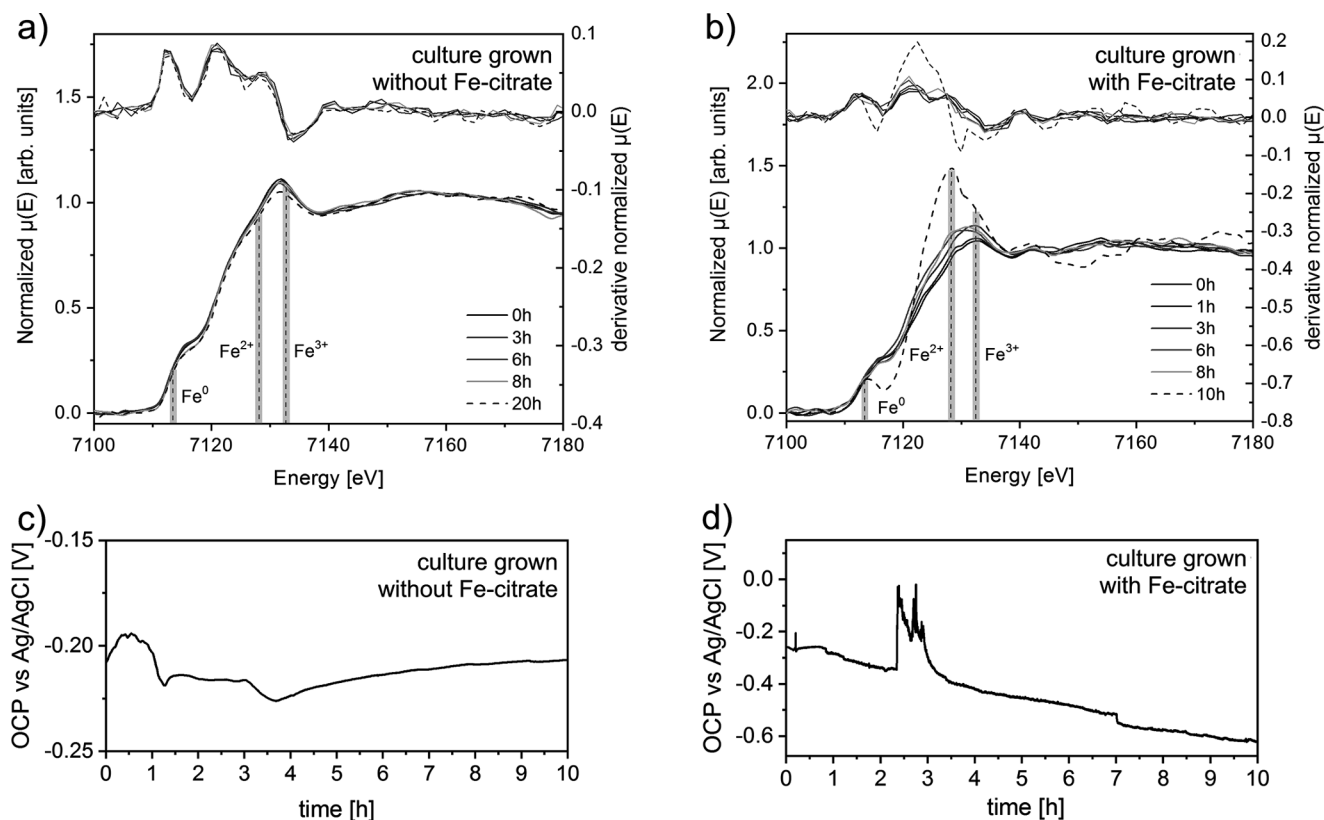


Fig. 2. XANES time series and their derivative spectra collected on thin iron films with backscattered fluorescence detection and OCP monitoring results a), c) CN32 cultivated in minimal media (CN32) b), d) CN32 cultivated in minimal media with addition of 16 g/l iron citrate (FeCi), vertical dashed lines correspond to the peak positions determined from the reference measurements.

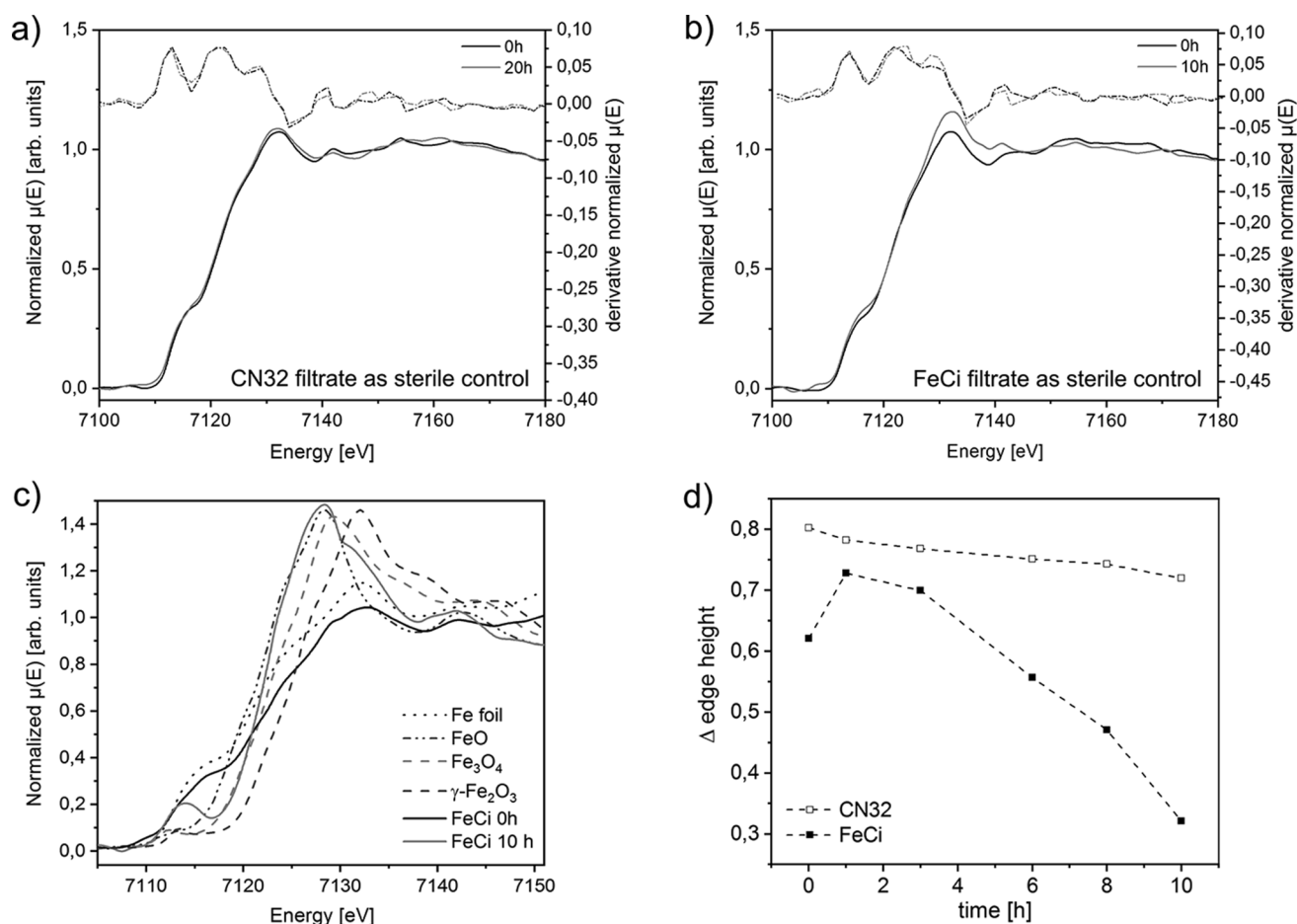
citrate (FeCi) are presented in Fig. 2. The XANES spectra of the sample incubated without Fe-citrate is characterized by a constant edge height of the metallic iron signal at 7112.4 eV until the eighth hour of the measurement. Since the edge height is directly proportional to the amount of detected iron in the beam, the edge height difference plotted over time presented in Fig. 3 d indicates that no significant dissolution or oxidation of the metallic iron took place in this time period. As seen in Fig. 2 c the OCP signal is initially fluctuating around  $-0.225$  mV and stabilizes with a slight anodic shift at later phases of the measurement. The last spectra collected after 20 h shows a minute decrease in the metallic edge without any significant changes in the oxidation states of the native oxides.

As seen in Fig. 2 b, when the same experimental procedure is applied to collect the time series with bacteria adapted to metabolize Fe (III) (FeCi) even within the first collected XANES spectra differences were observed in comparison to the CN32 culture, indicating the presence of significant amounts of  $Fe^{2+}$ - and/or  $Fe^{3+}$ -species. The maximum absorption energy range (white-line) increasingly develops a shoulder peak at 7128.0 eV together with the broad peak positioned at 7131.5 eV. The increase of intensities of both peaks continues until the eighth hour of measurement with a slight decrease in the edge height of the metallic iron signal. This indicates that the metallic film is undergoing oxidation and both Fe(II) and Fe(III) species are present in the oxide film [8]. Between 8 to 10 h, a sudden change in the white-line was observed and a negative shift of the edge position indicating a Fe (II) dominated composition of the surface film. Interpreted together with the spectra collected on reference samples presented in the overlay in Fig. 3 c, the surface film can be described as a mixture rich in  $FeO/Fe(OH)_2$  and further trivalent oxides and hydroxides. Due to the observed decrease in the metallic edge, the spectral detection of dissolved ferrous ions in the electrolyte at the immediate vicinity of the surface cannot be

completely ruled out. The changes in the surface chemistry are also reflected in the time trace of the open circuit potential. For the sample incubated with the culture grown in the presence of Fe-citrate, the OCP decreases from  $-0.25$  V at the beginning, to  $-0.62$  V after ten hours approaching the free corrosion potential of iron in chloride containing neutral electrolytes [17], indicating that the thin iron film is undergoing uniform corrosion. The electrochemical signal is not lost within the first ten hours of measurement assuring that the iron film is not completely dissolved.

Filtrates of the growth media are used as sterile controls to avoid any artifacts stemming from the metabolic products produced by the microorganisms during the cultivation. The XANES spectra collected on thin iron films in contact with the sterile media for the same duration as in the time series experiments are presented in Fig. 3 a and b. For both sterile controls, no significant changes in the metallic edge and the white-line position and intensity were observed.

In the case of the samples incubated with the CN32 culture and both sterile controls, the 80 nm thickness of the metallic bulk iron signal might have dominated the absorption signal hindering the observation of the minute changes in the outermost oxide layers in contact with the electrolyte. With the culture grown with the addition of Fe-citrate, the thinning of the metallic film due to the accelerated dissolution and its simultaneous conversion to ferrous oxy-hydroxides resulted in the rapid change in the XANES spectra. This observation is confirmed by the calculation of the absorption change from before and behind the edge energy (Fig. 3 d). The decrease in the edge height difference in the FeCi experiment supports the observed dissolution or thinning of the iron film due to bacterial activity. Interesting is also the slight initial increase of the iron signal within the first hour. The sample incubated with the culture grown without Fe-citrate on the other hand has undergone a steady but minute dissolution.



**Fig. 3.** XANES spectra collected on thin iron films in sterile filtrate of a) CN32, b) FeCi culture with backscattered fluorescence detection, c) comparison of the initial and final XANES spectra of iron films in FeCi culture with selected references d) edge height calculated from absorption before and above the edge for both cultures from conducted in situ measurements.

The microbial reduction of different ferric oxide/oxy-hydroxide species has been investigated mostly in suspensions by means of optical spectroscopy techniques such as FTIR and application of different assays [18,19]. The advantage of the in situ XANES is the transferability of the methodology to different metals and technical alloys, for which the synthesis of representative particle suspensions has not been established yet. Moreover, data acquisition at the same area throughout the experiment allows a direct comparison of the time resolved spectra. The consensus in the literature is that the reductive dissolution releases Fe(II) ions and  $\text{OH}^-$  into the immediate vicinity of the electrode, which can alter the local pH [20] and create environments of low Fe(II) solubility [9]. Furthermore, the growth of the biofilm limits the diffusion of Fe(II) ions. The presence of carbonates and phosphonates in the medium creates an environment favoring the formation of green rust as a mixed Fe(II)-Fe(III) hydroxide intermediate [21]. The comparison of the XANES spectra of the Fe sample incubated with the FeCi culture and the reference spectra indicates the formation of a Fe(II)-rich, iron oxy-hydroxide surface film. The fact that the process is sustained without any passivation could be explained with the presence of an electrically conducting and porous surface layer, which does not hinder the further corrosion of the underlying iron film. The presence of such conductive hydrogel like film was proposed by Stratmann and Hoffmann as a transformation product of lepidocrocite [22] under reducing conditions. Thus, our results agree very well with the literature. For clarifying the observed increase of the electrochemical activity with the culture grown in Fe(III) containing media, ongoing work is focusing on the metabolic and regulatory pathway analysis.

#### 4. Conclusions

In situ electrochemical XANES was used to monitor the micro-biologically induced changes in the passive film chemistry on model thin iron films. In comparison to *Shewanella* spp. grown in iron-free medium (CN32), cultivation in the presence of Fe(III) (FeCi) resulted in accelerated corrosion of the metallic iron film, which led to the appearance of a strong oxidic white-line at much shorter incubation duration. This effect most probably is due to an enhanced electrochemical activity of bacteria cultivated in Fe(III) containing medium overcoming the initial lag phase that regularly grown bacteria need to adjust onto utilizing iron oxides as electron acceptors. The continuous decrease of the OCP to the free corrosion potential of iron in neutral electrolytes supports the XANES findings that a constant dissolution and oxidation of the metallic iron film took place during incubation with the culture grown in the presence of Fe(III). Further microbiological studies are necessary to describe the metabolic and/or genetic changes leading to the observed increase of the electrochemical activity of the iron reducing *Shewanella putrefaciens* when cultured with abundant availability of Fe(III) ions.

#### CRediT authorship contribution statement

**Nina Wurzler:** Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Jan David Schutter:** Methodology, Validation, Writing - review & editing. **Ralph Wagner:** Methodology, Validation, Writing - review & editing.



**Matthias Dimper:** Methodology, Writing - review & editing. **Dirk Lützenkirchen-Hecht:** Methodology, Supervision, Writing - review & editing. **Ozlem Ozcan:** Conceptualization, Supervision, Visualization, Writing - review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

Authors gratefully acknowledge the DELTA Synchrotron Facility for beamtime allocation and DELTA machine group (Dortmund) for providing synchrotron radiation reliably. Furthermore, we are thankful to Yasser A. Shokr (Institute of Experimental Physics, Freie Universität Berlin) for technical support with the deposition of iron films.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.elecom.2020.106673>.

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