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# Combined geochemical and electrochemical methodology to quantify corrosion of carbon steel by bacterial activity

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ABSTRACT

The availability of respiratory substrates, such as $H_2$ and Fe(II,III) solid corrosion products within nuclear waste repository, will sustain the activities of hydrogen-oxidizing bacteria (HOB) and iron-reducing bacteria (IRB). This may have a direct effect on the rate of carbon steel corrosion. This study investigates the effects of *Shewanella oneidensis* (an HOB and IRB model organism) on the corrosion rate by looking at carbon steel dissolution in the presence of $H_2$ as the sole electron donor. Bacterial effect is evaluated by means of geochemical and electrochemical techniques. Both showed that the corrosion rate is enhanced by a factor of 2–3 in the presence of bacteria. The geochemical experiments indicated that the composition and crystallinity of the solid corrosion products (magnetite and vivianite) are modified by bacteria. Moreover, the electrochemical experiments evidenced that the bacterial activity can be stimulated when $H_2$ is generated in a small confinement volume. In this case, a higher corrosion rate and mineralization (vivianite) on the carbon steel surface were observed. The results suggest that the mechanism likely to influence the corrosion rate is the bioreduction of Fe(III) from magnetite coupled to the $H_2$ oxidation.



## 1. Introduction

Disposal of high-level nuclear waste (HLW) in deep geological repositories is increasingly considered as a reliable solution in many countries. In France, for example, the current option explored is to store the vitrified HLW in stainless steel containers, conditioned in carbon steel overpacks which are then emplaced in a deep underground repository (about 500 m deep) in an argillaceous formation (claystone). This is known as a multi-barrier system, designed to ensure long-term radionuclide confinement. One of the purposes of the multi-barrier system is to prevent water circulation around the metallic packages, thus preventing corrosion in water-saturated conditions.

However, knowledge about steel corrosion processes, especially over a long time period, must still be expanded to ensure that geological disposals will remain safe over a period of several hundred thousand years. The main issues related to steel corrosion are the influences of physico-chemical conditions (e.g. water saturation, pressure, temperature, pH, redox potential), and consequently microbial activity on the durability of the different metallic packages.

Several studies reveal the presence of microorganisms in most of the deep clay formations already investigated, such as the Callovo-Oxfordian argillite and Opalinus clay [1,2]. Therefore, an impact of the microbial activity can be expected with respect to the various phenomena that may occur within the repository, such as (i) radionuclide migration through clay formations (including effects of biofilms); (ii) build-up of the gas phase by microbial gas production; and (iii) Microbiologically Influenced Corrosion (MIC) or biocorrosion [3,4] which is discussed in this study.

Energetic substrates and nutrients are available to support microbial activity under geological conditions. Nutrients may be present either as soluble species in the groundwater or in minerals (solid-associated forms). Among the energetic substrates, $H_2$ is expected to be one of the most efficient substrates (acting as an electron donor) [5]. It can be produced by radiolytic dissociation of water or by anoxic aqueous metallic corrosion [6,7]. Moreover, Fe(III) from clay minerals [8,9] and corrosion products, such as magnetite ($Fe_3O_4$) [10–14], could be a significant electron acceptor for anaerobic microbial respiration (by dissimilatory processes). The availability of such substrates may sustain the development of hydrogen-oxidizing bacteria (HOB) and iron-reducing bacteria (IRB), which in turn could have an impact on geochemical and corrosion processes in deep geological environments.

Several studies have dealt with the impact of sulfate-reducing bacteria (SRB) [15–18] on corrosion processes. In contrast, the impact of the

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IRB species have been only marginally investigated. Their role in biocorrosion is still under debate; either an inducing or an inhibitory effect by formation of a protective biofilm on metal surface has been hypothesized [19–21]. Recent studies have investigated the impact of IRB species on metallic corrosion processes under geological disposal conditions [22–24]. The IRB can use Fe(III) from magnetite or other Fe(III) (hydr)oxides as electron acceptor in the presence of $H_2$ as electron donor. An alteration of the (hydr)oxide layers with a possible reactivation of the corrosion process may thus occur as a consequence of the Fe(III) bacterial respiration [22].

This study investigates the effect of the HOB and IRB activities on the corrosion rate of carbon steel in the presence of $H_2$ as the sole electron donor. These investigations are supported by geochemical and electrochemical techniques. Geochemical analysis allows the monitoring of the metal dissolution and the formation of Fe(II,III) solid corrosion products during the bacterial oxidation of $H_2$ produced by corrosion. Local electrochemical techniques allow to generate a high $H_2$ concentration for bacterial metabolism and then to probe the bacterial reaction in terms of modification of the local potential.

## 2. Materials and methods

### 2.1. Bacterial culture

The *Shewanella oneidensis* strain MR-1 (ATCC 700550™) was chosen as a model of IRB and HOB. Cultures were obtained aerobically at the beginning of the stationary growth phase in a Luria Bertani Broth (LB) medium (5 g $L^{-1}$ NaCl, 10 g $L^{-1}$ tryptone, 5 g $L^{-1}$ yeast extract) after 24 h at 30 °C under sterile conditions. Bacterial cells were harvested from the LB medium by centrifugation (4000 rpm for 20 min), washed once with sterile minimal medium (M1) and then inoculated in the batch reactors (initial concentration $10^8$ cells $mL^{-1}$ counted by epifluorescence method with LIVE/DEAD® *Bac*Light™ kit). The chemically defined minimal medium (M1) was prepared according to Kostka and Nealson (1998) [25]. However, minor modifications were made to the composition [22] in order to obtain a representative solution of the groundwater found in the argillaceous formations for geological disposal in France. The final composition is shown in Table 1.

**Table 1**
M1 minimal medium composition.

| Compound | Concentration |
|---|---|
| $(NH_4)_2SO_4$ | 9 mM |
| $Na_2SeO_4$ | 11 μM |
| HEPES | 17 mM |
| $NaHCO_3$ | 2 mM |
| $K_2HPO_4$[a] | 0.5 mM |
| $KH_2PO_4$[a] | 0.3 mM |
| $CoSO_4.7H_2O$[b] | 5 μM |
| $NiSO_4.6H_2O$[b] | 5 μM |
| NaCl[b] | 10 μM |
| $H_3BO_3$[c] | 45 μM |
| $ZnSO_4.7H_2O$[c] | 0.8 μM |
| $Na_2MoO_4.2H_2O$[c] | 3 μM |
| $CuSO_4.5H_2O$[c] | 0.2 μM |
| $MnSO_4.H_2O$[c] | 1 μM |
| $MgSO_4.7H_2O$[c] | 0.8 mM |
| $CaCl_2.2H_2O$[c] | 0.4 mM |
| $FeSO_4.7H_2O$[c] | 4 μM |
| Arginine[d] | 0.11 mM |
| Glutamate[d] | 0.13 mM |
| Serine[d] | 0.19 mM |
| Nicotinic acid[e] | 0.08 mM |
| Thiamine-HCl[e] | 0.01 mM |
| Biotine[e] | 0.40 μM |

[a] Phosphate buffer solution.
[b] Metals supplement solution.
[c] Basal salts solution.
[d] Amino acid solution.
[e] Vitamins solution.

The pH was adjusted to ca. 7 with NaOH and then the medium was sterilized by autoclaving (120 °C for 20 min), except for the thermolabile components (e.g. amino acids) which were filter-sterilized (0.22 μm) and added to the autoclaved medium.

### 2.2. Carbon steel coupons

Corrosion studies were performed with low carbon steel coupons A37 supplied by the French Alternative Energies and Atomic Energy Commission (CEA). They contain 0.12% C, 0.22% Si, 0.62% Mn, 0.008% Al, 0.012% S, 0.012% P, 0.02% Ni, 0.03% Cr, 0.04% Cu, 0.005% Co and <0.005% Ti. Carbon steel is a corrosion-allowance material which is expected to have nearly-uniform corrosion in a reducing environment, and has been therefore considered as a candidate material for packages used in the geological disposal.

The cylindrical coupons were laterally insulated from the solution by a diallyl phthalate glass-fiber resin (Presi) in order to expose only an active surface of 0.8 $cm^2$. Then, the coupons were polished with 600 grit SiC abrasive paper and sterilized with ethanol by sonication for 15 min prior to the experiments.

### 2.3. Batch experiments

All experiments started under strictly sterile conditions in batch reactors at 30 °C under an anaerobic atmosphere. Both abiotic and biotic conditions were investigated.

#### 2.3.1. Geochemical techniques

The geochemical experiments were performed in triplicate in 140 mL of M1 medium under an $N_2/CO_2$ (90:10%) atmosphere. The corrosion reaction was monitored as a function of time by gas and solution analyses. The aqueous Fe concentration was analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Varian, VISTA-MPX) after 0.02 μm filtration and 2% (v/v) $HNO_3$ acidification. The analysis of $H_2$ in the headspace was carried out by Micro Gas Chromatography (Varian, CP-4900) using a thermal conductivity detector with $N_2$ as carrier gas. The total $H_2$ concentration was calculated as the sum of the concentrations in the gas and the aqueous phases (determined using Henry's law).

#### 2.3.2. Electrochemical techniques

The electrochemical measurements were performed with a homemade Scanning Electrochemical Microscope (SECM) [26,27] in a 4-electrode cell configuration elaborated specifically for anaerobic and sterile conditions. The double wall glass cell was equipped with the carbon steel working electrode (WE1) placed in the bottom of the cell, and with a platinum probe of 50 μm in radius acting as a second mobile working electrode (WE2) for generating $H_2$. The cell was sealed with a Teflon cover and the platinum WE2 probe was placed through a nitrile flexible support on the cover, perpendicular to WE1, in a way to allow its displacement for the measurements. The WE2 microelectrode was positioned with the help of motorized stages driven by a motion controller (Newport) with Labview software. Both working electrodes used as reference a saturated calomel electrode (SCE) and a platinum grid as counter electrode.

The disassembled cell and the platinum WE2 probe were sterilized prior to the experiments with 70% ethanol solution for 20 min; then rinsed with sterilized water in a laminar flow chamber and finally UV-irradiated for 15 min. The lugging capillary (for holding the reference electrode) and the platinum grid counter electrode were sterilized in autoclave. The cell was assembled under sterile conditions and 230 mL of M1 medium was introduced and deaerated for 2 h under an $N_2$ atmosphere. The $N_2$ flow was maintained at the cell headspace (near to the liquid surface) so as to ensure the anaerobic conditions during the whole electrochemical measurements. A supplementary $N_2$