Bioelectrochemistry 97 (2014) 69–75

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Influence of hydrogen-oxidizing bacteria on the corrosion of low carbon steel: Local electrochemical investigations

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ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 14 June 2013
Accepted 4 October 2013
Available online 12 October 2013

Keywords:
Biocorrosion
Carbon steel
Hydrogen-oxidizing bacteria (HOB)
SECM
Local electrochemical impedance spectroscopy

ABSTRACT

Low carbon steel has been considered a suitable material for component of the multi-barrier system employed on the geological disposal of high-level radioactive waste (HLW). A non negligible amount of dihydrogen (H_2) is expected to be produced over the years within the geological repository due to the anoxic corrosion of metallic materials and also to the water radiolysis. The influence of the activity of hydrogen-oxidizing bacteria (HOB) and iron-reducing bacteria (IRB) on carbon steel corrosion is considered in this study because of the high availability of energetic nutriments (H_2, iron oxides and hydroxides) produced in anoxic disposal conditions. Local electrochemical techniques were used for investigating the activity of IRB as a promoter of local corrosion in the presence of H_2 as electron donor. A local consumption of H_2 by the bacteria has been evidenced and impedance measurements indicate the formation of a thick layer of corrosion products.

1. Introduction

The safe disposal of radioactive waste is a major concern for the nuclear energy industry. The high level radioactive waste (HLW) should be maintained for years in deep clay formations in order to prevent the migration of radionuclides. Thus, many different kinds of materials such as carbon steel, stainless steel, glass, concrete and clay are employed on the waste repository aiming to act as a multi-barrier system [1]. However, the anoxic corrosion of the metallic materials is expected due to the changes in the environmental conditions around the buried structures such as resaturation of the repository with time. In this context, corrosion products like iron oxides (i.e. magnetite, Fe_3O_4) or hydroxides, and dihydrogen (H_2) are also expected to be formed according to the following reactions (Eqs. (1)–(8)) [1]:

In aerobic condition

$$4Fe + 3O_2 \rightarrow 2Fe_2O_3 \quad (1)$$

$$4Fe + 6H_2O + 3O_2 \rightarrow 4Fe(OH)_3 \quad (2)$$

$$2Fe + H_2O + 3/2O_2 \rightarrow 2FeO(OH) \quad (3)$$

In anaerobic condition

$$3Fe + 4H_2O \rightarrow Fe_3O_4 + 4H_2 \quad (4)$$

$$Fe + 2H_2O \rightarrow Fe(OH)_2 + H_2 \quad (5)$$

$$3Fe(OH)_2 \rightarrow Fe_3O_4 + 2H_2O + H_2 \quad (6)$$

Mineral transformation and phase transition

$$4Fe_2O_3 + Fe \rightarrow 3Fe_3O_4 \quad (7)$$

$$2Fe(OH)_3 + 4Fe + 2H_2O \rightarrow 2Fe_3O_4 + 5H_2 \quad (8)$$

The production of dihydrogen poses two major problems. On the one hand, it represents a significant threat to the repository when accumulated for a long time in the surrounding clay because it may damage the barrier properties of the geological formation, affecting the safety of the repository. On the other hand, dihydrogen also represents a new energy source for microbial growth, especially in such anoxic environments with low content of biodegradable organic matter.

Several studies have already pointed out the possibility of microbiological life in the deep geological disposal [2–5], and both biological activity and biofilm formation may influence the metallic corrosion rate. These phenomena are usually known as microbiologically influenced corrosion (MIC), which can represent a huge problem

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http://dx.doi.org/10.1016/j.bioelechem.2013.10.003

when promoting local metal dissolution by different mechanisms which vary with the microbial species and chemistry of the colonized metal surface [6]. For instance, in such confined environment, some iron-reducing bacteria (IRB) are able to use simultaneously dihydrogen as electron donor and ferric iron as electron acceptor for their anaerobic respiration [7,8]. In this study, *Shewanella oneidensis* was chosen as an IRB and hydrogen-oxidizing bacteria HOB model microorganism. It is a facultative anaerobic bacterium [9] able to use different compounds as electron donor, such as lactate, formate, pyruvate, amino acids and also dihydrogen [10]. According to El-Naggar et al. [11] the respiration rate of *S. oneidensis* strain MR-1 is 2.6×10^6 electrons per cell per second using lactate as electron donor. In addition, some mechanisms of IRB species using electron transfers from solid substrates have already been described in the literature, such as:

- the use of mediators which can act as electron shuttles transferring electrons from the cells to the acceptors compounds [12,13];
- the direct contact of cells with solid substrate through multiheme cytochromes at the external membrane [14];
- the use of conductive intracellular filaments (i.e. nanowires) [11].

It is well-known that the presence of microorganisms can influence the corrosion rate [6,15–19], but is not well elucidated if IRB are able to induce local corrosion. The overall influence of microorganism on corrosion mechanism is still under debate since it depends on many factors [6].

Electrochemical techniques such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were already proved to be powerful tools for corrosion investigations. Valuable parameters can be gathered about the kinetics of interfacial processes from the measurements of current or potential. However, these results are obtained as an average behavior of the whole interface, which render difficult the elucidation of the local corrosion mechanisms [20–23]. Therefore, the use of local techniques is needed to describe the interface reactivity.

The objective of this study is to characterize the electrochemical interface by both local and global techniques during biocorrosion of low carbon steel in the presence of *S. oneidensis* used as model of IRB and HOB. Scanning electrochemical microscopy (SECM), which has been widely described by Bard et al. [24–26] and subject of numerous reviews [27–29], and local electrochemical impedance spectroscopy pioneered by Isaacs et al. [21,30] were used as electrochemical techniques. Carbon steel has been considered as candidate material for the multi-barrier system due to the low corrosion rate under reducing conditions. In a previous study [31], corrosion products, iron oxides (e.g. magnetite) and H_2 were shown to favor the hydrogen-oxidizing IRB development in the context of active corrosion of metallic radioactive waste containers. Moreover, the size of bacterial cells is in the range of a few micrometers to a few tens of micrometers. Such dimension makes possible to perform local electrochemical measurements even in the biofilm environment. A microelectrode can also be used for generating locally H_2 in the close vicinity of the bacterial cells.

2. Experimental

2.1. Bacterial culture

The cultures of *S. oneidensis* strain MR-1 (ATCC 700550™) were obtained aerobically at the beginning of the stationary growth phase in Luria Bertani Broth (LB) medium (5 $g\cdot L^{-1}$ NaCl, 10 $g\cdot L^{-1}$ tryptone, 5 $g\cdot L^{-1}$ yeast extract) after 24 h at 30 °C. *S. oneidensis* 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 electrochemical reactor (initial concentration of 10^8 cells$\cdot mL^{-1}$ counted by THOMA counting chamber). The chemically defined minimal medium (M1) was prepared according to Esnault et al. [31]. The composition consisted in a mixture of 9 mM $(NH_4)_2SO_4$, 2 mM $NaHCO_3$, 0.8 mM $MgSO_4\cdot7H_2O$, 0.4 mM $CaCl_2\cdot2H_2O$, 45 μM H_3BO_3, 10 μM NaCl, 4 μM $FeSO_4\cdot7H_2O$, 5 μM $CoSO_4\cdot7H_2O$, 5 μM $NiSO_4\cdot6H_2O$, 3 μM $Na_2MoO_4\cdot2H_2O$, 11 μM Na_2SeO_4, 1 μM $MnSO_4\cdot H_2O$, 0.8 μM $ZnSO_4\cdot7H_2O$, 0.2 μM $CuSO_4\cdot5H_2O$, 17 mM HEPES buffer, amino acids (0.11 mM arginine, 0.13 mM glutamate, 0.19 mM serine), and vitamins (0.08 mM nicotinic acid, 0.01 mM thiamine-HCl, 0.40 μM biotine). 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). For local impedance experiments this medium was prepared without phosphate ions in its composition.

2.2. Carbon steel coupons

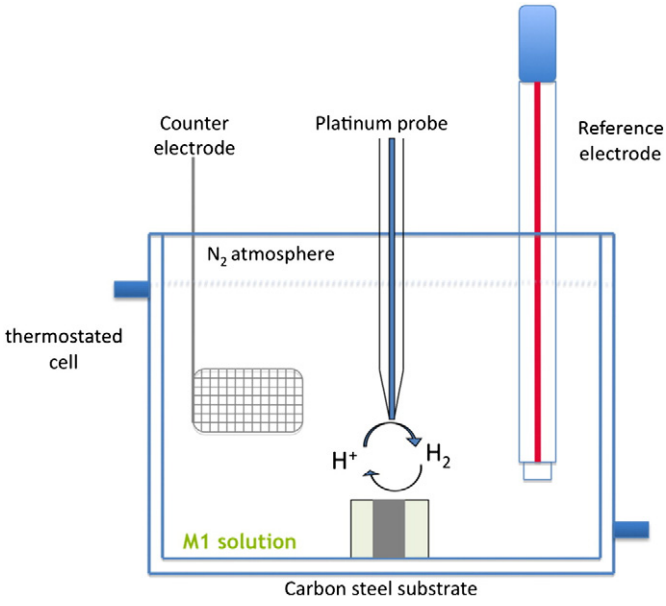
Low carbon steel coupons A37 (0.12% C, 0.22% Si, 0.62% Mn, 0.008% Al, 0.012% S and P, 0.02% Ni, 0.03% Cr, 0.04% Cu, 0.005% Co and <0.005% Ti) were obtained from the CEA (Commissariat à l'Energie Atomique et aux Energies Alternatives — France). Rod-shaped coupons (1 cm in diameter) were laterally insulated with a cathaphoretic paint and then molded in a diallylphthalate glass-fiber resin (Presi), leaving a disk area of 0.79 cm^2 exposed to the solution. Prior to each experiment, the electrode was polished with emery paper (P600 grit SiC) and sterilized with ethanol by sonication for 15 min.

2.3. Electrochemical experiments

2.3.1. Scanning electrochemical microscopy (SECM)

Preliminary experiments for the detection of H_2 were performed in a 0.5 M sulfuric acid solution with a platinum electrode of 0.5 cm in diameter as substrate and a platinum microelectrode of 20 μm in radius.

For the experiments related to the study of carbon steel corrosion, the electrochemical measurements were performed with a home-made scanning electrochemical microscope (SECM), which consisted in a 4-electrode cell configuration depicted in Scheme 1, elaborated specifically for anaerobic and sterile conditions. A double-wall glass cell thermostated at 30 °C allowed the carbon steel working electrode (WE1) to be placed in the bottom of the cell in a face up position. The reference and the counter electrodes consisted in a saturated calomel reference electrode (SCE) and a platinum gauze, respectively. The cell was closed with a Teflon cover where a flexible nitrile bracket was adapted. Throughout this flexible support, a platinum tip of 50 μm in diameter, acting as a second mobile working electrode for generating



Scheme 1. Sketch of the SECM setup used in this study.