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Influence of respiratory substrate in carbon steel corrosion by a Sulphate Reducing Prokaryote model organism

Leonardo T. Dall'Agnol, Cristina M. Cordas, José J.G. Moura *

REQUIMTE, Departamento de Química, Centro de Química Fina e Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

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ABSTRACT

Sulphate Reducing Prokaryotes (SRP) are an important group of microorganisms involved in biocorrosion processes. Sulphide production is recognized as a fundamental cause of corrosion and nitrate is often used as treatment. The present work analyses the influence of respiratory substrates in the metal, from off-shore installations, SRP influenced corrosion, using *Desulfovibrio desulfuricans* ATTC 27774 as model organism, since this can switch from sulphate to nitrate. Open Circuit Potential over 6 days in different conditions was measured, showing an increase around 200 and 90 mV for the different media. Tafel plots were constructed allowing E_{corr} and j_{corr} calculations. For SRP in sulphate and nitrate media E_{corr} values of −824 and −728 mV, and j_{corr} values of 2.5 and 3.7 μAcm^{-2}, respectively, were attained indicating that in nitrate, the resultant corrosion rate is larger than in sulphate. Also, it is shown that the equilibrium of sulphide in the solution/gas phases is a key factor to the evolution of corrosion Nitrate prevents pitting but promotes general corrosion and increases the corrosion potential and iron dissolution 40 times when compared to sulphate. Our results demonstrate that nitrate injection strategy in oil fields has to be considered carefully as option to reduce souring and localized corrosion.

1. Introduction

Corrosion is a natural process that leads to the deterioration of metals and alloys, causing release of the metal ions to the environment. It is an electrochemical phenomenon that occurs due electrons transfer mechanisms, in the presence of an electrolyte, in which the most common electron acceptor is oxygen, despite that, in acid conditions, protons may also play a final role electron acceptors [1,2].

Microbiologically Influenced Corrosion (MIC) or biocorrosion has been recognized as an important category of corrosion almost 50 years ago. Microbes can influence the deterioration of metals by a variety of ways, reflecting their physiological diversity [2]: (i) production of aggressive/corrosive metabolic products towards the protective layer or the metal itself; [3] secretion of enzymes that promote reduction processes at cathodic sites; (iii) degradation of chemical compounds that inhibit or enhance corrosion; (iv) production of Exopolymeric Substances (EPS) or biofilm development that alter the conditions at the metal surface.

A biofilm is a complex structure mainly composed by water (95%), bacteria, EPS—polysaccharides, proteins, lipids—corrosion products and metal ions [4,5]. These biopolymers can be classified as capsular (if linked to the cell surface by non-covalent interactions) or lime (if weakly associated to the cell surface). After attachment the biofilm is considered to have an important role on the resistance to biocides and antibiotics, acting as a chemical barrier against the diffusion of substances towards the microorganisms at the metal surface [6]. Some studies have already established a relationship between biofilm EPS and metal ion chelation in the biocorrosion process [7,8].

Sulphate Reducing Prokaryotes (SRP) are a morphologically and taxonomically diverse group (*Archaea* and *Bacteria*) being the most studied microorganisms associated to biocorrosion in both aquatic and terrestrial environments [9]. SRP performs dissimilatory reduction of sulphur compounds such as sulphate, sulphite and thiosulphate into sulphide. Some species from the *Desulfovibrio* genus (*Desulfovibrio desulfuricans*, for example) can use nitrate as alternative respiratory substrate. It has been reported that high concentration of nitrite inhibits sulphate reduction. Nitrite is also considered a key factor to other microorganisms that out-compete with SRP, being frequently considered as a strategy to control oil field souring [10]. Although they were historically classified as anaerobic microorganisms, today it is known that some genera tolerate oxygen and even grow in its presence, which reinforces its ubiquity around the globe [11].

SRPs have been implicated pitting corrosion process of ferrous metals in several anoxic habitats and its activity is of great concern to many industrial operations, in particular, oil and gas industries (O&G) [12]. The presence of hydrogenase enzyme and also the presence of iron sulphide have been indicated as key factors of steel deterioration by SRP according to the cathodic depolarization theory. Hydrogenase catalyses the reversible oxidation of hydrogen and is present in all SRP. Moreover, it is possible to establish a direct electron transfer between the enzyme and steel surface, since hydrogenase can remain active in biofilms for months even if the bacteria are not viable

* Corresponding author. Tel.: +351 21 294 83 82; fax: +351 21 294 85 50.
E-mail addresses: lt.dallagnol@campus.fct.unl.pt (L.T. Dall'Agnol), cristina.cordas@fct.unl.pt (C.M. Cordas), jose.moura@fct.unl.pt (J.J.G. Moura).

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[13,14]. Besides other molecules like flavin groups and the MtrC protein family (multihaem cytochromes) have been implicated in extracellular electron transfer [15,16].

The objective of this study is to compare the influence of the respiratory substrate(s) in order to understand: i) the role of the sulphide vector; ii) the influence of nitrate treatment on the metabolism of SRP; and iii) the consequences to the biocorrosion progression in carbon steel, using electrochemical, weight loss (WL) and surface analysis (Scanning Electron Microscopy) techniques.

2. Material and methods

2.1. Bacterial strain and growth conditions

For the development of the model organism and standardization of growth conditions, cells culture of *D. desulfuricans* ATCC 27774 were grown in two semi-defined culture media, Vitamin (VMN) Sulphate and Vitamin Nitrate [17], which differ only in the electron acceptor substrate. VMN base was composed of (g/l distilled water): KH_2PO_4, 0.5; NH_4Cl, 1.0; $CaCl_2 \cdot 2H_2O$, 0.04; sodium lactate, 6.0; sodium citrate, 0.3; casamino acids, 2.0; tryptone, 2.0; modified Wolfe's mineral elixir (0.1% volume/volume) and vitamin solution (0.2% volume/volume). The sulphate complement was (g/l distilled water): Na_2SO_4, 4.5; $MgSO_4 \cdot 7H_2O$, 0.06; $FeSO_4 \cdot 7H_2O$, 0.004. The nitrate complement was (g/l distilled water): $NaNO_3$, 2.4; $MgCl_2 \cdot 6H_2O$, 0.05; $FeCl_2 \cdot 4H_2O$, 0.003. The Vitamin solution was composed of ($g\,l^{-1}$ distilled water): riboflavin, 0.1; nicotinic acid, 0.25; thiamine, 0.3; pentatonic acid, 0.3; pyridoxine, 0.3; cyanocobalamin, 0.025; ascorbic acid, 1; biotin, 0.005. The composition of the modified Wolfes Elixir was (g/l distilled water): Nitrilotriacetic acid, 1,5; $MgSO_4 \cdot 7H_2O$, 0.06; $MnSO_4 \cdot H_2O$, 0.5; NaCl, 1; $FeSO_4 \cdot 7H_2O$, 0.1; $CoSO_4 \cdot 7H_2O$, 0.1; $NiCl_2 \cdot 6H_2O$, 0.1; $CuCl_2 \cdot 2H_2O$, 0.1; $ZnSO_4 \cdot 7H_2O$, 0.1; $CuSO_4 \cdot 5H_2O$, 0.01; $AlK(SO_4)_2 \cdot 12\text{-}H_2O$, 0.01; H_3BO_3, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $Na_2SeO_3 \cdot 5H_2O$, 0.001.

All the chemicals were p.a. grade purchased form Sigma-Aldrich. After weighting and mixing all components, the pH value was adjusted with KOH (5 M) to a final value of 7.45–7.55. The medium was then degassed with argon flux to ensure anoxic conditions and sterilized by autoclaving for 20 min at 120 °C and 1 atm pressure. The vitamin solution, sterilized by filtration (0.22 μm cellulose, Millipore) was added after, just before the inoculation in a concentration of 0.2% (volume/volume) of the final volume of the culture.

The inocula were performed with cell suspensions in exponential phase in the rate of 10% (volume/volume) in the presence of a Bunsen burner to ensure sterile conditions. The growth curve was made in the presence of a metal coupon of carbon steel St52–3 N simultaneous to the electrochemical assay at 30 °C. The planktonic cell growth was followed by optical density at 600 nm, using a Shimadzu® UV–VIS Spectrophotometer model UV-1800, as an indicative parameter of the metabolic phase and cell availability for the biofilm formation. Aliquots were taken each 8 h of culture for the first 24 h and them once per day (48, 72, 96, 120 and 144 h) in order to further evaluate the lactate, sulphate and nitrate consumption by High Performance Liquid Chromatography (HPLC), ICS3000 (DIONEX) using an Ionpac AS11 HC column and an AG11 HC pre-column. Sulphide production was quantified by a colorimetric assay modified from elsewhere (the modified method is available in the Supplementary Material S1) [18]. The pH was also monitored each 24 h with a CRISON microph 2001 (Crison Instruments). All studies described involved triplicates of the cultures.

2.2. Exopolymeric substances extraction

For the extraction of the colloidal and capsular EPS a protocol described elsewhere was used [19]. The samples were grown in 5 L VMN Sulphate and VMN Nitrate for 3 days at 30 °C until they reached a minimum cell concentration of 10^8 cells. Then the cultures were centrifuged at 8000 ×*g* for 15 min. The supernatant was collected and filtered with a 0.22 μm pore cellulose membrane to remove contaminant cells. All samples were dialyzed against deionized water for 16 h and then twice for 2 h changing the water.

2.3. Electrochemical experiments with model organism and EPS

The working electrode (WE) was a carbon steel St52–3 N coupon (geometric area of 0.4 cm^2), composed of the following elements with mass ratio of 0.2% C, 1.6% Mn, 0.55% Si, 0.025% S, 0.025% P. The WE were prepared by polishing in an oxide-silicon carbide sandpaper grit 600 (P1200) and flushed with dry air in order to avoid any alteration in the oxide layer of the surface. Sterility was assured with one hour exposure to UV light (253.7 nm) in an Airflow workstation. The counter electrode (CE) was a graphite rod. The reference electrode (RE) was a saturated calomel electrode (SCE), which was in contact with the system through a bridge tube that was filled with the same electrolyte of the experiment.

All electrochemical measurements were registered using an Autolab/PGSTAT30 potentiostat/galvanostat, in a one compartment (1 L) MultiPort™ Corrosion Cell (Gamry®) in a three electrode configuration. The data acquisition was performed using the GPES version 4.9 software (AUTOLAB, EcoChemie B.V).

The electrolyte was the culture media VMN Sulphate or Nitrate. The sterile electrolytes were purged with sterile humidified argon for 1 h prior to the experiment. To maintain strict anoxic conditions and avoid contaminations, a permanent flow of sterile humidified argon was kept in the headspace of the cell to ensure a positive pressure during all tests. For the nitrate negative control, a final concentration of 10 μg/mL Ampicillin and 50 μg/mL of Kanamycin were added daily to prevent contaminations. All experiments were performed at 30 °C to simulate the same temperature at the oil field platform from where the metal coupons were obtained. In sulphate, cellulose membranes with 0.22 μm pore sizes were used in order to evaluate the influence of biofilm attachment to the corrosion process. In sulphate incubations, two different flow pressures were tested: a Low Pressure Ratio (LFR), equivalent to an argon flow at the headspace of 0.3 L per minute; and a High Pressure Ratio (HFR), equivalent to an argon flow at the headspace of 3 L per minute. Also, in sulphate cultures a 2 M zinc acetate trap was placed in the gas exit to precipitate the H_2S produced.

For the model organism Open Circuit Potential (OCP) and Cyclic Voltammetry (CV) tests were performed. All potential values are referenced to SCE. The OCP were measured for a period of 144 h (6 days) with an interval time of 90 s. The parameters for CV were as follow: equilibrium time, 5 s; scan rate, 1 mV/s (quasi-stationary state condition); E_i, −0.750; E_f, −0.750 V; Upper vertex, −0.350 V; Lower vertex, −1 V.

For the EPS only CV assays were performed in the same conditions as referred for biofilm experiments.

2.4. Weight loss and surface analysis experiments

For the weight loss (WL) and surface analysis by scanning electron microscopy [20], VMN Sulphate and VMN Nitrate were the growth media. Metal plates (rectangles) with measures of 20 × 10 × 2 mm were used as coupons. The St37 carbon steel used is composed of the following elements with mass ratio of 0.17% C, 1.4% Mn, 0.045% S, and 0.045% P. The plates were cleaned as described above. In both cases there was no degrease so the material has the same treatment as the ones used in the oil field. A hole of 1.5 mm area was drilled to hang the coupon using a nylon thread, having each coupon a total area of 3.7 cm^2. All coupons were weighted in a precision scale just before being hanged at the rubber stopper and placed in an empty 100 mL anaerobic bottle. After closing the bottles, with 4 coupons each, they were exposed for 1 h to UV light (253.7 nm) in an Airflow workstation to assure sterility of the coupons. A previously prepared medium was