Bioelectrochemistry 101 (2015) 14–21

Contents lists available at ScienceDirect

Bioelectrochemistry

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

Electron mediators accelerate the microbiologically influenced corrosion of 304 stainless steel by the *Desulfovibrio vulgaris* biofilm

Peiyu Zhang [a,1], Dake Xu [b,1,*], Yingchao Li [a], Ke Yang [b], Tingyue Gu [a,**]

[a] Department of Chemical and Biomolecular Engineering, Institute for Corrosion and Multiphase Technology, Ohio University, Athens, OH 45701, United States
[b] Institute of Metal Research, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 16 May 2014
Accepted 20 June 2014
Available online 1 July 2014

Keywords:
Biofilm
Biocorrosion
SRB
Electron transfer
Electron mediator

ABSTRACT

In the microbiologically influenced corrosion (MIC) caused by sulfate reducing bacteria (SRB), iron oxidation happens outside sessile cells while the utilization of the electrons released by the oxidation process for sulfate reduction occurs in the SRB cytoplasm. Thus, cross-cell wall electron transfer is needed. It can only be achieved by electrogenic biofilms. This work hypothesized that the electron transfer is a bottleneck in MIC by SRB. To prove this, MIC tests were carried out using 304 stainless steel coupons covered with the *Desulfovibrio vulgaris* (ATCC 7757) biofilm in the ATCC 1249 medium. It was found that both riboflavin and flavin adenine dinucleotide (FAD), two common electron mediators that enhance electron transfer, accelerated pitting corrosion and weight loss on the coupons when 10 ppm (w/w) of either of them was added to the culture medium in 7-day anaerobic lab tests. This finding has important implications in MIC forensics and biofilm synergy in MIC that causes billions of dollars of damages to the US industry each year.

1. Introduction

Microbiologically influenced corrosion (MIC), which is primarily caused by the activity or metabolic products of microbes, is becoming an increasingly important branch of research in metal corrosion. MIC was a primary suspect for the leak of the Alaska oil pipeline at Prudhoe Bay in 2006 [1]. It caused a major spike in world crude oil prices and drew considerable public attentions to potential environmental damages a pipeline leak could bring. Up to 20% or more of all corrosion losses may be attributed to MIC and this means billions of dollars each year in the United States alone [2]. Apart from financial losses, pipeline leaks can cause environmental disasters. Release of flammable liquids and gases or the highly toxic gas H_2S is also a major safety concern. Oxygen is typically removed from pipelines using oxygen scavenger because it is highly corrosive. However, anaerobic corrosion remains a severe threat. CO_2, H_2S corrosion and acetic acid corrosion are examples of conventional (i.e., abiotic) chemical corrosion that threaten the pipeline industry [3]. Some oxidants such as sulfate do not cause corrosion abiotically because their reduction requires biocatalysis. They become a threat when corrosive biofilms are present. Sulfate reducing bacteria (SRB) are the most common bacteria associated with anaerobic MIC because of the wide availability of sulfate in various aqueous environments such as seawater that is typically used in water injection to increase oil reservoir pressures [4,5]. SRB are anaerobic bacteria that can use sulfate as the terminal electron acceptor to produce hydrogen sulfide [6]. They can tolerate exposure to oxygen for a period of time, but without any growth [7].

1.1. Theory and hypothesis

Xu and Gu in 2011 [8] argued that SRB MIC is primarily caused by the favorable thermodynamics of extracellular elemental iron (Fe^0) oxidation coupled with intracellular sulfate reduction in the SRB cytoplasm under biocatalysis. This process generates energy during sulfate respiration by SRB. It is classified as Type I MIC that requires electron transport from outside the cell across the cell wall into the cytoplasm [9]. Venzlaff et al. investigated electron transfer in this type of MIC by SRB [10].

Type II MIC is caused by microbes that secrete corrosive metabolites that are oxidants, including protons and (undissociated) organic acids [9]. These oxidants attack metals such as Fe^0 extracellularly without biocatalysis by removing electrons from Fe^0. This is why acid corrosion can occur without a biofilm in conventional chemical corrosion. In fact, abiotic acetic acid corrosion in the oil and gas industry is an important research topic [11]. A biofilm secretes locally high concentrations of these oxidants causing localized attacks. Corrosion by hydrogen sulfide released by SRB metabolism belongs to Type II MIC. However, this is unlikely the dominant mechanism in many SRB attacks on carbon steel. Xu and Gu designed a carbon starvation experiment in 2011

* Corresponding author. Tel.: +86 24 23971880.
** Corresponding author. Tel.: +1 740 593 1499.
E-mail addresses: xudake@imr.ac.cn (D. Xu), gu@ohio.edu (T. Gu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bioelechem.2014.06.010
1567-5394/

[12]. They first grew *Desulfovibrio vulgaris* biofilms in the same full-strengthen ATCC (American Type Culture Collection) 1249 culture medium and then replaced the medium with fresh culture media that had 0%, 90%, 99%, and 100% carbon source removed. They found that starved, but not completely carbon source starved, biofilms became more aggressive against carbon steel. This observation supported the Type I MIC argument for SRB MIC. Both Type I MIC and Type II MIC are electrochemical in nature because an oxidation reaction (e.g., iron oxidation) and a reduction reaction (e.g., reduction of sulfate or proton) can be separated. However, the two basic MIC types are fundamentally different. In Type II MIC, electrons are exchanged on the metal surface locally without cross-cell wall electron transfer that is essential in Type I MIC. Type II MIC is similar to conventional chemical corrosion [13], except that the oxidants are secreted by microbes.

The classical cathodic depolarization theory (CDT) was applicable to MIC by hydrogenase-positive SRB [3]. These SRB use dissolved hydrogen as electron carrier. Hydrogen is a well-known electron carrier in biofilm electrochemistry [14]. There are, however, other electron transfer mechanisms that are not covered by CDT. Thus, Gu and Xu proposed a more general theory called Biocatalytic Cathodic Sulfate Reduction (BCSR) theory using the two reactions below to explain Type I MIC by SRB [12].

$$4Fe \rightarrow 4Fe^{2+} + 8e^{-} \text{ (Iron dissolution) } E^{o\prime} = -447 \text{ mV} \quad (1)$$

$$SO_4^{2-} + 9H^{+} + 8e^{-} \rightarrow HS^{-} + 4H_2O \text{ (BCSR) } E^{o\prime} = -217 \text{ mV} \quad (2)$$

$E^{o\prime}$ is defined as the reduction potential (also known as equilibrium potential or redox potential) at 25 °C, pH 7, and 1 M solutes (or 1 bar partial pressure for gases). The redox reaction combining Reactions (1) and (2) has a cell potential of +230 mV, which yields a negative Gibbs free energy of reaction ($\Delta G^{o\prime}$) under the conditions defined for $E^{o\prime}$. This suggests that sulfate corrosion of carbon steel can occur spontaneously with energy production. However, sulfate reduction is a kinetically retarded process that requires biocatalysis. Reaction (2) depicts a simplified sulfate reduction process. The actual process follows the APS (adenosine phosphosulfate) pathway inside the SRB cytoplasm that consumes 8 electrons for each sulfate reduced as shown in Fig. 1 [15]. Reaction (2) should not be interpreted as a net proton consumption reaction. For example, it was found that there is no net production of vectorial or scalar protons in sulfate reduction in *D. vulgaris* [16].

Because the electrons released by extracellular Fe^0 oxidation cannot freely "swim" in an aqueous environment from outside a SRB cell to the SRB cytoplasm, an elaborate cross-cell wall electron transfer chain is needed [3]. Microbes capable of this kind of electron transfer are known as electrogens [14,17,18]. Electrogenic microbes have been utilized in microbial fuel cells (MFCs) to produce electricity by extracting electrons from organic carbon oxidation [19]. Extracellular electron transfer (EET) describing the electron transport between electrodes and microbial cells plays a critical role in MFC research [20,21]. The mechanisms of EET are mainly classified into two categories — direct electron transport (DET) that relies on the specific protein-based structures such as *c*-type cytochromes or pili, and mediated electron transport (MET) that utilizes redox-active chemical mediators to facilitate the electron transfer indirectly [18,22]. Sherar et al. [23] found that in the absence of organic carbon (electron donor) in the culture medium, a SRB isolated from an oil well grew pili linking the cells with a carbon steel coupon surface to harvest energy. In this case, pili were used to transfer extracellular electrons released by iron oxidation for sulfate reduction inside SRB. When the culture medium has a utilizable organic carbon, it will diffuse into the cytoplasm to donor electrons without EET. Electrons released from organic carbon oxidation are transferred from the biofilm covering the anode to the anode surface of an MFC. This electron transfer direction is opposite to that in MIC, but biofilm electron transfer is often reversible. For example, some MFCs use a biocathode instead of an oxygen cathode [24,25]. In this case, electrons are transferred from the anode via an external circuit to the cathode and then from the cathode to the biofilm covering the cathode. Thus, the electron transfer direction between the cathode and the cathodic biofilm is exactly the same as in MIC, i.e., from outside the cell to the cytoplasm inside the cell by crossing the cell wall (Fig. 2). The knowledge gained from electron transfer in MFC investigations is directly relevant to MIC [14].

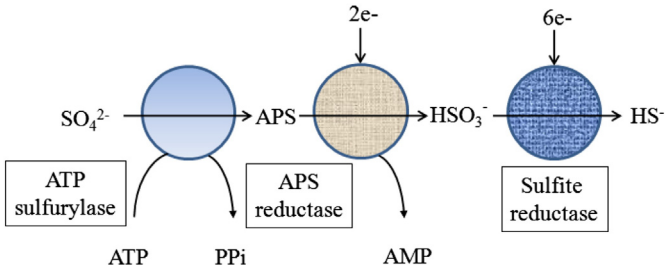

Fig. 1. APS pathway for sulfate reduction by SRB. Figure was drawn after Voordouw [15]. APS is the substrate of ATP (adenosine triphosphate) sulfurylase. AMP (adenosine monophosphate) is produced by hydrolysis of ATP to AMP and pyrophosphate (PPi).

Electron mediators are soluble compounds that are redox active. They are electron carriers. They absorb electrons and release electrons at different locations. Exogenous electron mediators such as ferrocyanide are those chemicals that are added to a microbial system, while endogenous mediators are those that are secreted by the cells themselves. Riboflavin, quinine-containing humic acids, phenazines, and flavin adenine dinucleotide (FAD) are common endogenous mediators [20,26–28]. In a synergistic biofilm consortium, it is possible that a non-electrogenic microbe may play the role of supplying electron mediators to an electrogenic species [18]. This may be the reason why biofilm consortia typically perform better in MFCs than pure-strain biofilms [29].

Humic acids, which have quinine structures, can be reduced to hydroquinone to work as electron donors for anaerobic respiration [30]. Flavins, such as riboflavin and FAD, are another type of well-known mediators. Riboflavin is the water-soluble vitamin B_2, which is a precursor to FAD and flavin mononucleotide (FMN) in biosynthesis [31]. FAD and FMN are enzyme cofactors with the ability to accept electrons and they are involved in the catalysis of many redox reactions [32]. The ability of electron binding by flavins is mainly determined by the structure of their isoalloxazine ring [32,33]. Molecular hydrogen (H_2) is also a "universal" electron carrier because H^+ can absorb an electron [34]. The classical CDT theory actually implies electron transfer using H_2 by hydrogenase-positive SRB [35]. However, it is not applicable to hydrogenase-negative SRB that use other electron carriers or use direct electron transfer (DET) without electron mediators [9].

Electron transfer is often a bottleneck in electricity generation by MFCs. Intensive research has been carried out to improve electron transfer. One method is the introduction of electron mediators or the use of biofilms that produce endogenous electron mediators. Inspired by this, this work investigated the hypothesis that electron mediators could play a major role in accelerating MIC.

2. Material and methods

2.1. Chemicals, coupons, bacterium and cultivation

BioReagent-grade riboflavin and FAD were purchased from Sigma-Aldrich (St. Louis, MO, USA) as electron mediators for MIC tests. They were used separately at a level of 10 ppm (w/w). Coin-shaped 304 stainless steel coupons with a top exposed surface area of 1.1 cm^2 were prepared by sequential polishing with 180, 400, and 600 grit abrasive papers and cleaned with 75% (v/v) isopropanol afterwards. Coupons were dried under ultraviolet (UV) light for at least 15 min before use. Three coupons were added into each 125 ml anaerobic vial (Catalog No.