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Discerning different and opposite effects of hydrogenase on the corrosion of mild steel in the presence of phosphate species

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ABSTRACT

Mild steel coupons were exposed to hydrogenase in a 10 mM phosphate solution. Control coupons were covered by a layer of vivianite. The injection of hydrogenase caused a fast increase in the open circuit potential; this increase depended on the amount of hydrogenase injected and increased from 8 mV for 30 μL hydrogenase to 63 mV for 80 μL. The presence of enzyme resulted in a thicker deposit: high amounts induced the accumulation of corrosion products. Hydrogenase that was deactivated by air revealed a protective effect: non-degradation was observed. In contrast, hydrogenase that was denatured by heat provoked an important deposit of corrosion products with a heterogeneous, cracked structure. The study showed that the action of hydrogenase is not linked to its regular enzymatic activity but to a balance between the protective effect of its protein shell and the electrochemical action of its iron-sulphur clusters. Depending on the operating conditions, hydrogenase can either enhance or mitigate the formation of a corrosion layer on mild steel.

1. Introduction

Sulphate-reducing bacteria and thiosulphate-reducing bacteria (SRB/TRB) are the most clearly identified causes of anaerobic microbially influenced corrosion (MIC) of steels in natural environments [1–7]. Several mechanisms have been proposed to explain anaerobic MIC by SRB and TRB [8–15]. As far as SRB are concerned, the most often evoked mechanism is based on the production of sulphide ions by the metabolic reduction of sulphates. Sulphide ions react with iron ions, forming iron sulphide which deposits on the material surface and catalyses the reduction of proton:

$$H^+ + e^- \Rightarrow 1/2H_2 \qquad \text{or} \qquad H_2O + e^- \Rightarrow 1/2H_2 + OH^- \tag{1}$$

Several studies have discussed the efficiency of iron sulphides in catalysing proton reduction depending on the crystal state and the structure of the deposit [16–17]. Contrarily to what has been said sometimes in the past, the consumption of the final hydrogen, by SRB or other means, cannot have a direct effect on the corrosion rate [18]. In Eq. (1) the forward reaction of electron uptake by the proton is the rate-limiting step on steel surfaces in anaerobic environments. Consuming the final hydrogen product cannot consequently have any direct effect on the rate of electron extraction from the material. Consequently, consuming the final hydrogen cannot enhance the corrosion process. Consumption of hydrogen by SRB can only have an indirect effect by promoting the development of SRB on the material surface and enhancing the production of sulphide ions, for instance.

Some studies have demonstrated that there is a direct correlation between the presence of hydrogenase in SRB and corrosion [19–20], while it has also been claimed that a hydrogenase-negative strain of SRB is more corrosive than hydrogenase-positive strains [21]. Hydrogenases are a group of enzymes that catalyse the reversible oxidation of hydrogen (Eq. (1)) [22–23]. Hydrogenases are divided into three groups according to the composition of their active site [22,24]: [NiFe]-, [FeFe]-, and the Fe—S cluster-free hydrogenases (initially called metal-free and now renamed [Fe]-hydrogenase [25–26]). The [NiFe]- and [FeFe]-enzymes form the vast majority. [FeFe]-hydrogenases are known to have 100 times more H_2 production specific activity than [NiFe]-hydrogenases [27]. In the metabolic pathway, they transfer the electrons to specific redox partners (Med) like cytochromes, nicotinamide adenine dinucleotide (NAD^+) or ferredoxin (Fd_{Ox}). They also can use artificial mediators as electron acceptors. For instance, the [Fe]-hydrogenase from *Clostridium acetobutylicum* that was used in this work can exchange electrons with ferredoxin (natural partner) or methyl viologen (artificial mediator), both following the global reaction:

$$H_2 + Med_{Ox} \Leftrightarrow 2H^+ + Med_{Red} \tag{2}$$

Hydrogenases have been claimed to be involved in corrosion mechanisms either by being present inside bacterial cells or by being free after cell lysis [20,28]. Several studies have tried to elucidate the possible effect of free hydrogenases on the corrosion of steels and have proposed two kinds of mechanisms.

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1.1. Mechanism 1: catalysis of hydrogen consumption with involvement of phosphate species (Schematic 1)

A synergetic effect of hydrogenase and phosphate species on corrosion was first pointed out by Bryant and Laishley [29–30], who observed that hydrogenase increased the corrosion rate of carbon steel when used in a phosphate solution. These authors proposed a direct reaction between steel and phosphate ions:

$$3Fe^{\circ} + 4H_2PO_4^- \rightarrow Fe_3(PO_4)_2 + 3H_2 + 2HPO_4^{2-} \quad (3)$$

This mechanism was then reworked, demonstrating that phosphate species undergo a so-called cathodic deprotonation on steel surfaces [31]:

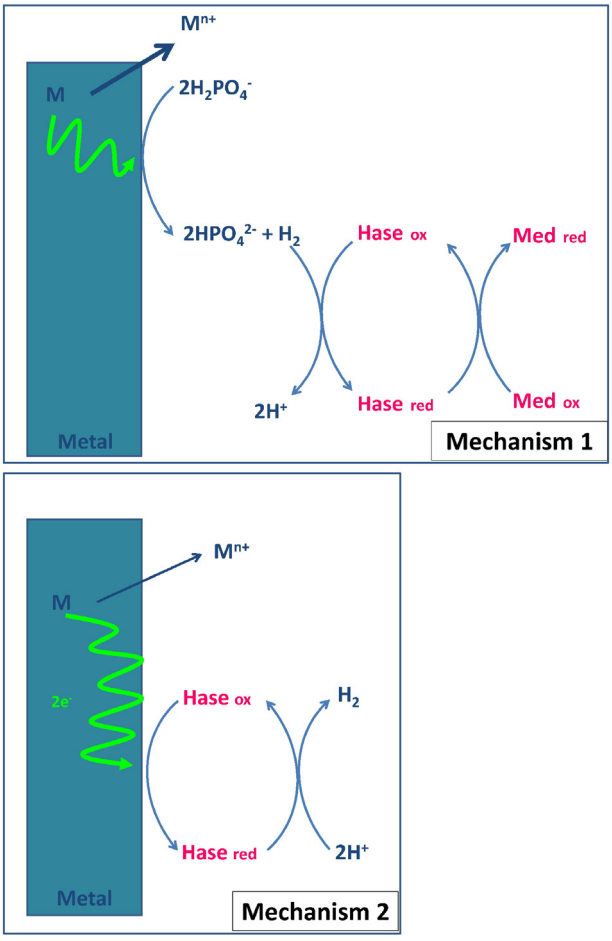
$$H_2PO_4^- + e^- \Leftrightarrow HPO_4^{2}- + \S H_2 \quad (4)$$

This reaction, coupled with acid equilibrium:

$$HPO_4^{2}- + H^+ \Leftrightarrow H_2PO_4^- \quad (5)$$

presents the phosphate species as an efficient homogeneous catalyst for the reduction of proton/water [8]. The cathodic deprotonation of phosphate species (Reaction (4)) is relatively fast on steel surfaces and not strictly limited by the forward electron uptake (as is Eq. (1)). On steel surfaces, Reaction (4) is a balanced reaction that can be shifted by the consumption of hydrogen. In this case, with significant concentrations of phosphate in the solution, the consumption of hydrogen can increase the rate of electron extraction from the material, and consequently increase corrosion. This process has been shown on mild steel using hydrogenase from *Ralstonia eutropha*, which catalysed the oxidation of hydrogen with NAD^+ as a final electron acceptor [32]. However hydrogenase can enhance corrosion following this mechanism only in the presence of two different compounds:

- a compound able to ensure a balanced cathodic deprotonation (like phosphate species, Reaction (4))
- a final electron acceptor, with which the enzyme is able to work (depends on the hydrogenase species).



Scheme 1. Mechanisms for hydrogenase action on steel corrosion in anaerobic phosphate medium; a) Mechanism 1: Catalysis of hydrogen consumption with involvement of phosphate species. b) Mechanism 2: catalysis of proton reduction by adsorbed hydrogenase. Hase is for hydrogenase in its reduced (red) or oxidized (Ox) form. Med is for mediator in its reduced (red) or oxidized (Ox) form.

1.2. Mechanism 2: catalysis of proton reduction by adsorbed hydrogenase (Schematic 1)

The second mechanism is based on the direct catalysis of proton reduction by adsorbed hydrogenase. The catalysis by adsorbed hydrogenase of direct electron extraction from different metals has already been demonstrated in the literature. Hydrogenases from *Thiocapsa roseopersicina* and *Lamrobacter modestohalophilus* have been shown to catalyse the oxidation of metals directly, without the need for a mediator [33]. Hydrogenases from *T. roseopersicina* and *Alcaligenes eutrophus* can use cadmium particles directly as electron donors to produce hydrogen or to reduce NAD^+. It has been assumed that this mechanism can accelerate metal dissolution and thus be a key to MIC processes [34]. Moreover, hydrogenases from *Methanococcus maripaludis* can use iron granules to produce hydrogen by a direct electron transfer [35]. As well, on pyrolytic graphite, hydrogenases from *Escherichia coli* are able to catalyse some electrochemical reactions which are only possible with a large overpotential in absence of catalyser [36]. Hydrogenase from *R. eutropha* (new name for *A. eutrophus*) adsorbed on stainless steel has also been claimed to create a direct cathodic reaction on stainless steel [37]. Nevertheless, in this case, because of the presence of both a final electron acceptor and phosphate buffer, significant involvement of Mechanism 1 may be suspected.

Catalysis of electron extraction by adsorbed hydrogenase has been evoked several times in the literature as a likely key step in anaerobic MIC. Nevertheless, to our knowledge, our previous work carried out with hydrogenase from *C. acetobutylicum* was the first experimental demonstration that hydrogenase increased the corrosion of steel [38]. In this study, experiments have been performed in the absence of any final electron acceptor other than protons and water. In this condition, hydrogenase cannot oxidise the hydrogen that results from the corrosion process. Consequently Mechanism 1 cannot occur and hydrogenase can act only via the direct catalysis of proton or water reduction.

The purpose of the current study was to progress in deciphering the fine mechanisms of hydrogenase action in the corrosion of mild steel. The high concentration of phosphate that was used in the previous study (100 mM) interfered with the results because of the large amount of vivianite that formed rapidly on the steel surface. Here, the experiments were performed with less concentrated phosphate solutions (10 mM). No other electron acceptor than proton and water was present in solution, neither natural redox partner (oxidised ferredoxin) nor artificial mediator, in order to avoid the occurrence of Mechanism 1.

2. Materials and methods

2.1. Chemicals and biochemicals

Solutions were prepared in deionised water (ELGA PURELAB, 10–15 MΩ·cm) with analytical grade chemicals: sodium dihydrogenophosphate (Prolabo), tris(hydroxyl-methyl) aminomethane (named