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Exacerbation of the mild steel corrosion process by direct electron transfer between [Fe-Fe]-hydrogenase and material surface

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ABSTRACT

The influence of [Fe-Fe]-hydrogenase from *Clostridium acetobutylicum* on the anaerobic corrosion of mild steel was studied and the use of a dialysis bag to contain the enzyme in the close vicinity of the electrode surface led to conclusive tests. Electrochemical measurements (open-circuit potential monitoring, corrosion rate evolution, impedance), and surface and medium analysis, all confirmed the strong effect of hydrogenase to exacerbate the corrosion process. Electrolysis performed at a cathodic potential proved that hydrogenase catalysed the electrochemical reduction of protons or water into dihydrogen by direct electron transfer, demonstrating the involvement of hydrogenase in cathodic depolarisation.

1. Introduction

Hydrogenases are produced by microorganisms from diverse phylogenetic classifications, such as dissimilatory metal reducing bacteria [1], fermentative bacteria [2] and methanogenic archaea [3], among others [4]. Hydrogenases, which are either present in bacteria or free in solution, have been identified as key proteins in microbially induced corrosion (MIC) phenomena [3,5–10] but this is still a controversial issue. Even though these enzymes are present in most of the microorganisms involved in corrosion, the participation of hydrogenases in a direct electron transfer mechanism has rarely been demonstrated. Otherwise, free enzymes are often assumed to be unstable but Chatelus et al. [11] have demonstrated that hydrogenases can be stable over months and that their activity does not depend on the presence of viable cells. Thus, bacteria (which contain hydrogenases) or free hydrogenases that are still attached to metallic surfaces may retain their catalysing capabilities for a long time. Yates et al. [12] have also shown that the hydrogen reaction can be enhanced not only by living cells but also by cell debris (including killed microorganisms and hydrogenase).

Hydrogenases are defined as oxidoreductases that have their redox potential at the same potential as the H_2/H^+ redox couple (−410 mV vs standard hydrogen electrode) at ambient standard conditions (25 °C, atmospheric pressure, all concentrations 1 M except for the pH of 7) [12]. In anaerobic conditions, hydrogenases catalyse the reversible reaction shown in Eq. (1) [13–17].

$$2H^+ + 2e^- \leftrightarrow H_2 \quad (1)$$

This reduction reaction, which is the driving force of anaerobic corrosion, is generally considered as the rate-limiting reaction in the corrosion process of steel. Two mechanisms of hydrogenase action on corrosion have been established [5,6,18]. The first involves a synergetic effect between hydrogenase and phosphates (or weak acids) in presence of a redox mediator [18–20]. The second mechanism proposed does not require a redox mediator and hydrogenase catalyses the reduction of protons or water by direct electronic transfer [5,6]. The ability of various purified redox enzymes to accept electrons directly from cathodic surfaces has been repeatedly demonstrated [3,7,21,22], and the particular involvement of free hydrogenase in metal corrosion has sometimes been proposed [3,5,6,11]. However, there is no clear demonstration, at this time, of corrosion process catalysis by direct electron transfer between the hydrogenase and the mild steel, that can be considered as a cathodic depolarisation. When investigating the impact of an electromethanogenic archaeon called *Methanococcus maripaludis (M.m.)* on the corrosion of iron granules, Deutzmann et al. discovered an apparently direct electron uptake explained by redox-active enzymes, such as hydrogenases [3]. This was demonstrated by using the wild-type strain of *M.m.* and a mutant strain with no hydrogenases. When Fe(0)-containing vials were amended with the mutant cell-free culture medium, the H_2 formation rate was equivalent to the abiotic control (51 ± 7.8 nmol electron equivalents (eeq)/h) whereas, with the wild type

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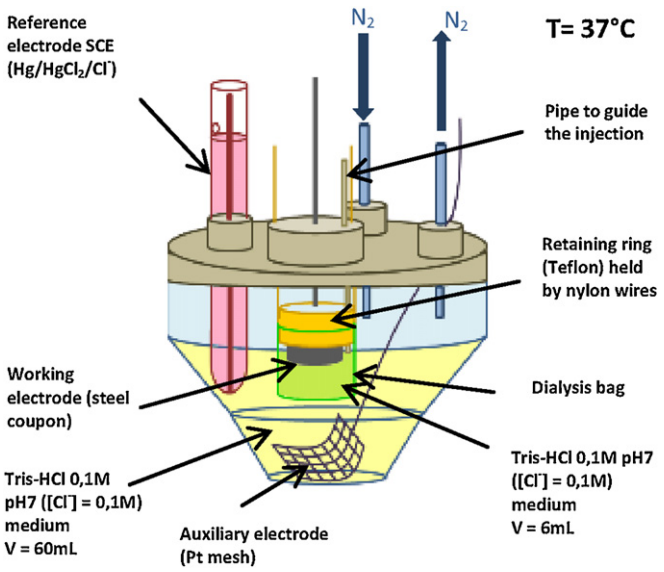

Fig. 1. Scheme of the experimental setup 2 with a dialysis bag.

cell-free culture medium, the formation rate of H_2 was more than 5 times higher (274 ± 49 nmol eeq/h). Thus, hydrogenases released from cells could be adsorbed on the Fe surface and, by direct electron transfer, could catalyse the H_2 formation. In their natural environment, release of hydrogenases in the medium by some microorganisms has been suggested to be accidental or to take place with the purpose of producing the small compounds required for the catabolism of others: development thanks to a sacrificial subpopulation [3]. Thus, the impact of microorganisms (such as IRB or SRB) on the corrosion process [23,24] could be directly due to the involvement of hydrogenases as free enzymes in solution rather than occurring only inside cells as was suspected so far [25]. The free hydrogenases could adsorb to an electron donor surface to catalyse the formation of H_2 and thus the corrosion process.

Because of their excellent properties (high efficiency, high specificity, low over-potential for H_2 oxidation, biodegradability), hydrogenases are emerging as good candidates in many processes, notably in biofuel cells. Hydrogenase coated electrodes have been investigated [21,26,27], often using a graphite-based electrode [27–30]. These technologies are also based on the idea that hydrogenases could be adsorbed on the surface of electrodes and could take electrons directly from conductive materials at this surface to catalyse the formation of H_2. Lukey et al. [28] adsorbed two different hydrogenases from *Escherichia Coli* as a film on a pyrolytic graphite edge electrode (at pH6, 30 °C) and showed that they were able to catalyse electrochemical reactions that usually required a large overpotential. But the efficiency of these coated electrodes is very limited, notably due to the inactivation of the hydrogenases because of the protein desorption [26] and/or deactivation/conformation change [30]. A new strategy is being explored to better immobilize hydrogenases on the electrode surface by orienting them [26,30].

Among the hydrogenases ([Ni-Fe]-hydrogenases, [Fe-Fe]-hydrogenases, [Ni-Fe-Se]-hydrogenases), [Fe-Fe]-hydrogenases were chosen for the present study because of their high activity in the reduction of protons (10- to 100-fold that of [Ni-Fe]-hydrogenases) [31]. [Fe-Fe]-hydrogenases, often found as monomers, contain a catalytic domain, called the H-cluster, and a variable number of Fe-S clusters thought to be involved in electron transfer [14,21,32]. The H-cluster of [Fe-Fe]-hydrogenase is composed of a 2Fe subsite covalently bound to a [4Fe-4S] sub-cluster.

In the present work, the [Fe-Fe]-hydrogenase from *Clostridium acetobutylicum (Ca)* was selected, as this bacteria is known to be the microorganism that most efficiently produces hydrogen from hexose [31,33]. Its catalytic domain is an arrangement of six iron atoms in the form of two groups: [4Fe-4S] and [2Fe], where the two iron atoms connected together constitute the active site. After the purification process, the hydrogenase was embedded in a buffered solution with low concentrations of dithionite and desthiobiotin. It has been demonstrated in a previous work that these molecules have an inhibiting effect on the corrosion process [34]. The concentration of the additional molecules, essential for the purification process, was optimized to allow a high activity of hydrogenase and a lower impact on the electrochemical response for corrosion tests simultaneously. While this previous work only studied the effect of additional molecules, the present one focused on the influence of hydrogenase on the anaerobic corrosion of mild steel, using a containment technique (dialysis bag) that concentrated the amount of enzyme around the surface. Electrochemical measurements (open-circuit potential monitoring, polarisation resistance, impedance and cathodic electrolysis) and surface and medium analysis were used to decipher the hydrogenase action in chloride neutral medium.

2. Materials and methods

2.1. Hydrogenase production and purification

Tris(hydroxymethyl) aminomethane (named Tris) was purchased from Acros Organic, and hydrochloric acid, sodium dithionite and desthiobiotin were from Sigma. The Tris-HCl medium was prepared in order to have a final concentration of 0.1 M of Tris in distilled water. The pH was adjusted to pH7 or pH8 by adding concentrate HCl solution and using mechanical stirring. Once the pH had stabilized, the Cl^- concentration was adjusted to 0.1 M using KCl.

The hydrogenases in this work were [Fe-Fe]-hydrogenases. They are not commercialized and were produced, extracted and purified from *Clostridium acetobutylicum* cells in a 0.1 M pH8 Tris-HCl medium at INSA, Toulouse. The purification process used and the measurement of their oxidation activity were as described in [35,36].

At the end of the purification process, no dithiothreitol (DTT) was added to the fraction of interest, thus avoiding the "polluted" effect for the electrochemical study of hydrogenase, as shown in a previous work [34]. The pure [Fe-Fe]-hydrogenase was recovered in a 0.1 M Tris-HCl medium containing dithionite and desthiobiotin at low concentrations. The hydrogenase solution was divided into aliquots, flushed with pure hydrogen and stored at 4 °C. The specific activities (measured on the day of injection into the electrochemical cell), concentrations and compositions of the hydrogenase solutions used in this work are reported in Table 1. Specific activities represent the catalytic activity per unit mass of protein (U/mg of enzyme).

Hydrogenase solutions 1 and 2 were from the same purification fraction. Hydrogenase solution 3 was derived from another purification fraction with an optimized composition of additional molecules (dithionite and desthiobiotin) [34].

2.2. Electrochemical cell setup

The experiments were performed with a three-electrode system in closed cells (Metrohm).

The working electrodes were 2-cm-diameter cylinders of S235JR mild steel from Descours-Cabaud, France (elemental composition by weight percentage: Fe balance, 0.17C, 1.4Mn, 0.55Cu, 0.03S,