## Direct Electron Transfer Between *R. eutropha* Hydrogenase and Steels: Possible Role In MIC

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Anaerobic corrosion of steels results from the association of the anodic reaction of iron dissolution and a cathodic reaction of water or proton reduction. Any phenomenon that is able to increase the rate of this cathodic reaction may enhance the corrosion process. When materials are immersed in seawater, or in other natural environments, they are covered by micro-organisms, which form a biofilm on their surface. In anaerobic conditions marine biofilms contain sulfate-reducing bacteria, which have been demonstrated to be able in some cases to drastically enhance corrosion rates, creating so-called Microbiologically Influenced Corrosion (MIC) [1]. Numerous different mechanisms have been suggested to explain anaerobic MIC of carbon steel in seawater. Even if it still remains discussed, several studies suggest that the presence of hydrogenase-positive SRB strains is required to observe MIC [2].

The purpose of the work was to determine whether hydrogenases are able to exchange electrons with steel materials creating a reduction reaction that could drive MIC. Hydrogenase from *Ralstonia eutropha* was used because it catalyzes the reduction of NAD^+ to NADH:

$$NAD^+ + H_2 \quad \leftrightarrow \quad NADH + H^+$$
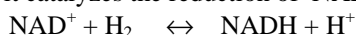
which allowed the reaction to be continuously monitored by UV-visible spectrophotometry [3].

In the first part of the work, spectroelectrochemistry was performed with a thin transparent cell equipped with a 316L stainless steel grid. Electrolyses at different potentials were performed from -0.60 V/SCE to -0.90 V/SCE by successive 50 mV steps. The phosphate buffer solution contained various NAD^+ concentrations (0 to 5 mM) and various hydrogenase activities (0 to 20 U/ml). For potential values from -0.75 to -0.90 V/SCE, NADH production rate in the presence of hydrogenase was significantly greater than without hydrogenase. Figure 1 gives the quantity of electricity consumed during each constant potential electrolysis. From -0.75 V/SCE to -0.90 V/SCE, the charges consumed with hydrogenase were greater than the charges consumed without hydrogenase.

In the second part galvanic coupling was performed between two XC48 carbon steel electrodes. Both electrodes were set up in a cell divided in two parts A and B by a dialysis membrane, each part containing phosphate buffer pH 8.0 and NAD^+ 5mM. Electrodes were electrically coupled through a 1 Ohm resistance, and current was followed as a function of time. For the experiments without hydrogenase, electrons flowed through the electrical circuit from electrode A to electrode B oscillating between -1 and 0 $\mu A/cm^2$. After 24 h no conversion of NAD^+ to NADH and no corrosion were observed.

When hydrogenase was present in compartment B the galvanic current indicated that electrons flowed through the electrical circuit from electrode B to electrode A, oscillating between 2 and 4 $\mu A/cm^2$ over 10 h. Consequently, electrode B globally behaved like an anode, but it presented local cathodic sites, because around 10% NAD^+ was converted to NADH. Optical micrographs showed very heterogeneous surfaces on both electrodes. Electrode A seemed to be significantly attacked. Electrode B showed a black non-conductive deposit composed of two types of crystals, as confirmed by scanning electron microscopy (Figure 2). X-Ray diffraction (XRD) indicated that the electrode B deposit was composed of vivianite and a double potassium/calcium phosphate.

These results demonstrated that hydrogenase can directly extract electrons from stainless steel and carbon steel, with a likely participation of phosphate ions. The discussion focus the importance of such a direct electron transfer for MIC initiation, and a new mechanism is proposed based on the direct catalysis by hydrogenase of the cathodic reaction.
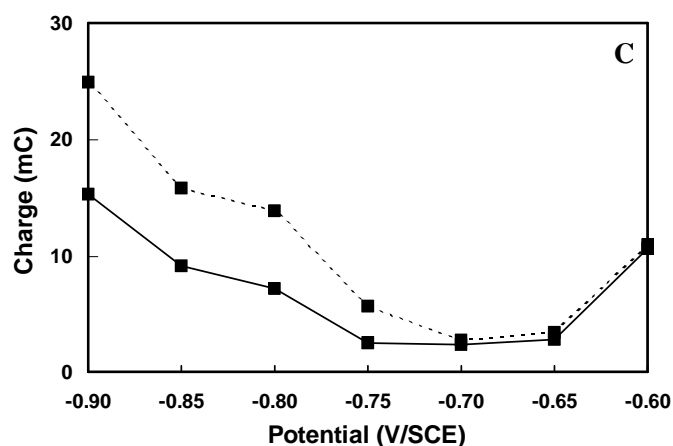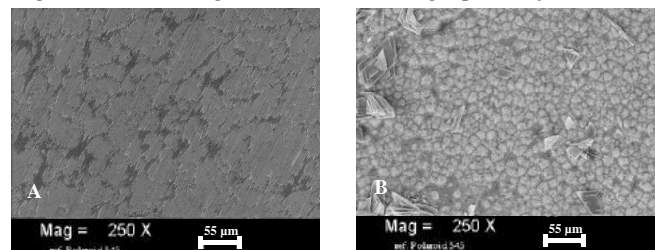

Fig. 1. Charges derived from the integration of the current-time curves recorded during successive electrolysis at constant potentials in a thin spectroelectro-chemical cell; continuous line: 2 mM NAD^+; doted line: 2mM NAD^+, 20 U/ml hydrogenase.

Fig. 2. Scanning electron micrographs of XC48

electrodes after 24h galvanic coupling through 1Ω resistance; phosphate buffer pH 8.0 contained 5 mM NAD^+, and 10 U/ml hydrogenase in compartment B.

References
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