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New evidences on the catalase mechanism of microbial corrosion☆

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Abstract

Changes on the oxygen reduction rate induced on aluminium brass by cell-free bacterial cultures of an isolate belonging to the genus *Pseudomonas* were studied in relation to the bacteria phase of growth and to the surface oxide layer composition after various electrochemical pre-treatments of the metal samples. Cultures isolated from the stationary phase of growth strongly influenced the oxygen reduction kinetics. Cathodic currents increased throughout the potential range tested when Cu_2O and CuO were present simultaneously in the surface film (so-called aged surfaces). In this case, the maximum increment (35%) was observed within the oxygen reduction limiting current region. On pre-oxidised surfaces, when the oxide film was composed mainly by CuO, the effect induced by stationary phase cultures was even higher, with the limiting current density increasing by almost 60%. On pre-reduced surfaces on the other hand, when only a submonolayer of Cu_2O was covering the surface, there was no effect as current density values were similar to those obtained in control experiments. Exponential phase cell-free cultures did not modify the limiting current values in any of the surfaces investigated. Results were in agreement with the participation of catalase as a bacterial catalyst for the oxygen reduction process. The normalised catalase activity from different stationary phase cell-free cultures ranged from 0.88 to 4.02 mg ml^{-1} U^{-1}, while there was no observable activity in exponential phase cultures. The incidence of the catalase mechanism in microbiologically influenced corrosion processes induced by aerobic biofilms is highlighted on the basis of the results obtained using metabolites from planktonic cells and their agreement with most of the experimental evidences so far reported by other authors.

Keywords: Oxygen reduction; Aluminum–brass; Surface films; Catalase; Microbiologically influenced corrosion

1. Introduction

It is well known that surfaces immersed in natural waters are readily colonised by microorganisms [1]. As a consequence of this colonisation and the subsequent development of microorganisms, the surface becomes covered by a biofilm. Biofilms are complex microbial populations composed by microbial clusters separated by channels and void spaces, which develop on virtually all immersed surfaces. Due to this ubiquity, biofilms are associated with many natural and industrial processes [2–4] and play a key role in the way how microorganisms influence corrosion processes [5,6].

Numerous reports have been written over the years describing a shift in the corrosion potential of different metals to the positive direction when exposed to natural waters [7–10]. This ennoblement has been related to the growth of aerobic microbial biofilms, although the mechanism governing the process remains obscure. There are however, clear evidences relating the electrochemical phenomena to the organisms' metabolism.

The electrochemical explanation to the ennoblement process is based on the increment of the oxygen reduction rate induced by the presence of bacteria. Mollica et al. [7] were one of the first to present results suggesting the existence of a close correlation between the respiratory electronic transport system (ETS) activity of biofilms and the changes in the oxygen reduction kinetics on stainless steel. In addition, Scotto et al. [9] showed that the potential ennoblement can be suppressed adding sodium azide (NaN_3) to the water. Schiffrin and de Sánchez [11] demonstrated the modification of the oxygen reduction kinetics by bacteria of the genus

☆ This work is dedicated to the memory of Dr. Vittoria Scotto who has greatly contributed to the study of Microbiologically Influenced Corrsion and who died recently.

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Pseudomonas, and proposed the role of bacterial metabolites as electrocatalysts. Similarly, it has been proposed that enzymes entrapped in the bacterial extracellular polymeric material could be responsible for the increment in cathodic currents [12]. The relation found between the depolarisation effect of biofilms and the extracellular polymeric substances produced by bacteria within the biofilm reinforced this idea.

Questions have been raised concerning the identification of bacterial metabolites which could participate in the catalytic effect. To our knowledge, the only enzyme ever proposed for which a mechanism has been presented is bacterial catalase. Busalmen et al. [13], were the first to show an increment of the cathodic currents when working with cell-free cultures of *Pseudomonas sp*., and depicted a mechanism concerning the coupling of the electrochemical oxygen reduction with the activity of the enzyme. The mechanism requires the electrochemical production of hydrogen peroxide during the oxygen reduction process, and involves electron recycling during the enzymatic decomposition of peroxide by catalase. Recently, the catalase mechanism has been tested by other authors on glassy carbon electrodes [14]. However, the occurrence of this mechanism on metals with technological applications remains unproved and needs to be explored.

Multiple forms of catalase are present in various microorganisms [15,16]. In Gram-negative bacteria, the production of several catalases has been reported in response to different stimuli. The exposure of *Escherichia coli* cells to H_2O_2 results in the expression of hydroperoxidase I (HPI), with catalase activity [15]. Similarly, the expression of an inducible catalase has been described in *P. putida* when cells were grown on roots producing active oxygen species [17]. In addition, when *E. coli* cells enter the stationary phase, a second type of catalase (HPII) is produced, whose expression is controlled at the transcriptional level [15].

Catalase is an integral component of the bacterial cells response to oxidative stress, and together with superoxide dismutase and alkyl hydroperoxidases is thought to limit the accumulation of reactive oxygen species [15]. Catalase reduces the intracellular concentration of hydrogen peroxide by catalysing its decomposition to water and oxygen as [15]:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (1)$$

A schematic representation of the proposed catalase mechanism, i.e. the coupling of reaction (1) with the oxygen reduction process on the surface, [13] is shown in Fig. 1. It is commonly accepted that oxygen reduction can proceed through a parallel mechanism involving the direct reduction via four electrons and a pathway where intermediate peroxide is formed. In this last case, oxygen electroreduction proceeds by the transference of two electrons, with the production of H_2O_2 as an intermediate compound, and then to hydroxyl ions by the transference of two more electrons [18]. On copper, Vázquez et al. [18] demonstrated that the second two electrons are readily transferred ($k_{III} \gg k_{IV}$ in Fig. 1), yielding low levels of H_2O_2 in solution. However, the same authors showed that, depending on the composition of the surface oxides, the electroreduction of peroxide can be inhibited, favouring its desorption to the solution ($k_{III} \ll k_{IV}$) [18]. The catalase mechanism is based on the enzymatic conversion of electrochemically produced H_2O_2 to water and oxygen in the proximity of the surface (reaction (1)). The oxygen generated by the enzyme can be electrochemically reduced in addition to the oxygen reaching the surface by diffusion from the bulk, resulting in higher cathodic limiting currents that can be attributed to the increase in the oxygen concentration [13].

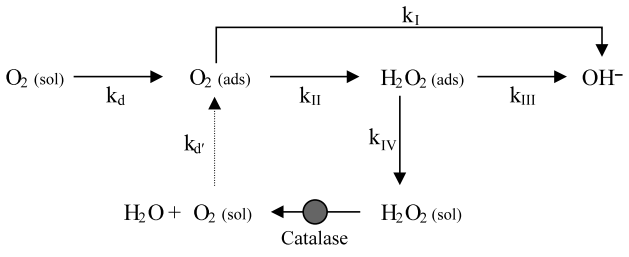

Fig. 1. The catalase mechanism of microbial corrosion. The electrochemical reduction of oxygen is coupled with the enzymatic decomposition of hydrogen peroxide to yield an autocatalytic cycle through which the reduction current density can be increased. k_n are the rate constant at step n. Species in solution (sol), or adsorbed to the surface (ads) are indicated.

Bacterial catalases are, in general, intracellular enzymes. However, the induction of an extracellular form of catalase in response to oxidative stress has been described in stationary phase cultures of *Bacillus subtilis* [16], being the production and excretion of the enzyme to the culture media dependent on the bacteria phase of growth.

In a previous paper [13], we reported the increase in cathodic current values both, in the presence of cultures of a *Pseudomonas sp*., and in the presence of cell-free cultures of the same bacteria, as compared to current values obtained in sterile media. Those results suggested the extracellular appearance of a bacterial metabolite with a catalytic effect on the oxygen reduction. Based on additional evidence the activity of the bacterial catalase was proposed to be coupled with the electrochemical production of hydrogen peroxide within an autocatalytic mechanism where the increase in oxygen reduction currents could be explained.

In this work, additional evidences are presented concerning the participation of catalase in the changes induced in the oxygen reduction rate on aluminium brass, a material widely used in the construction of heat exchanger tubes, when exposed to bacterial cell-free cultures of a wild strain of *Pseudomonas sp*. The influence of the phase of growth of bacterial cultures and

the chemical composition of the surface oxide films are evaluated.

Although evidences were obtained by developing in vitro experiments using metabolites present in filtrates of planktonic cultures, the idea of microbial catalase participating in the enhancement of corrosion of metals covered by natural biofilms is reinforced, and the agreement between the proposed mechanism and previously reported data is discussed.

2. Materials and methods

2.1. Biological material

Pure cultures of a wild strain of *Pseudomonas sp.* isolated from a heat exchanger tube refrigerated with seawater [13] were grown at 32 °C with continuous shaking in rich medium containing Lab Lemco (Merck) 0.1 g l^{-1}, yeast extract (Sigma) 0.2 g l^{-1}, and peptone (Sigma) 0.5 g l^{-1}, dissolved in artificial seawater (ASW) [13]. The growth of each culture was determined measuring absorbance at 600 nm (A_{600}). Cultures from both, the exponential and the stationary phase of growth, were harvested by centrifugation for 10 min at $10000 \times g$ in a Jouan BR4i refrigerated centrifuge. Cells were discarded. Supernatants were vacuum filtered using a 0.2 μm pore size membrane filter to obtain cell-free cultures. The absence of bacteria was verified by plating samples of the filtrate in solid rich medium prepared adding 1.5% agar to the liquid media described above.

2.2. Metal samples preparation and electrochemical measurements

Working electrodes were constructed from aluminium brass (ASTM B111) disks cut from condenser tubes, flattened, and mounted with fast curing acrylic resin on appropriate PVC holders with an electrical contact on the back. A saturated calomel electrode (SCE) ($E = -0.241$ V vs. NHE) was used as reference throughout the experiments. All the experiments were carried out using a LYP M5 Potentiostat (La Plata, Argentina).

Before each experiment, the electrode surface was smoothed with 600 grit emery paper. After polishing the metal surfaces were rinsed with distilled water and ethanol. Samples were later kept at open circuit potential for 15 min in stagnant conditions in ASW, letting the surface reach a steady-state value of the corrosion potential ($E_{corr} = -0.287 \pm 0.002$ V). These electrodes will be further referred to as aged samples. Pre-reduced and -oxidised surfaces were also investigated. For the experiments with pre-reduced surfaces, polished samples were polarised during 15 min to -1.0 V in deaereated ASW. Oxygen was removed by bubbling Ar during 10 min. For the experiments with pre-oxidised surfaces, polished samples were polarised during 3 min at 0.1 V (vs. E_{corr}) in aerated seawater. Air saturation was reached by bubbling air during 10 min.

Cathodic polarisation curves were performed using cell-free bacterial cultures as electrolyte. Cultures were previously aerated by bubbling air for 10 min. Aged and pre-oxidised samples were polarised in -0.01 V steps starting from the corrosion potential. Pre-reduced samples were polarised in the positive direction starting from -0.6 V (SCE) to prevent the formation of oxides before the experiments. Steady state current values were registered 30 s after applying each potential step.

Control polarisation curves for every surface condition were registered in non-inoculated culture media following the described procedures.

2.3. Catalase activity determination

Catalase activity was determined in cell-free cultures using a modification of the dicromate–acetic acid technique described by Shina [19]. Briefly, a sample of cell-free culture was mixed with a standard hydrogen peroxide solution to allow peroxide consumption during different periods of time. The enzymatic reaction was stopped by adding the dicromate–acetic acid reagent. The mixture was heated to reduce dicromate in the presence of the remaining H_2O_2, and cromic acetate was determined by measuring absorbance at 570 nm.

The rate constant (K) for the decomposition of H_2O_2 by catalase was determined as [19]:

$$K = (1/t)\log(C_0/C_t)$$

where C_0 is the initial concentration of H_2O_2 and C_t is the concentration of peroxide after t min. The initial reaction rate (K_0) was obtained plotting the dependence of K values with reaction time. The catalase content (C_{cat}) in the sample was expressed in terms of the pure crystalline catalase from *M. lysodeikticus* [20] specific activity (Kat_M: 230 mg ml s^{-1}) as:

$$C_{cat} = K_0/Kat_M$$

whereas a normalised catalase content (C_n) in the cell-free cultures was determined as the catalase content per absorbance unit at 600 nm of the cell culture [16]:

$$C_n = C_{cat}/A_{600}$$

3. Results

3.1. The influence of bacterial growth phase on the catalysis of oxygen reduction

Results in Table 1 showed that catalase activity could be detected in cell-free cultures from stationary phase

Table 1
Catalase activity determined in cell-free cultures of *Pseudomonas sp*. at the exponential and the stationary phase of growth

Culture no.	Exponential phase	Stationary phase		
		K_0 (min^{-1})	$C_{cat} \times 10^{-6}$ (mg ml^{-1})	$C_n \times 10^{-6}$ (mg ml^{-1} U^{-1})
1	ND	0.0167	1.21	0.88
2	ND	0.1043	7.55	4.02
3	ND	0.0379	2.74	2.66

The calculated initial reaction rate (K_0), the catalase content (C_{cat}), and the normalised enzymatic activity per absorbance unit of the bacterial culture (C_n) are listed for various cultures. ND, non-detected.

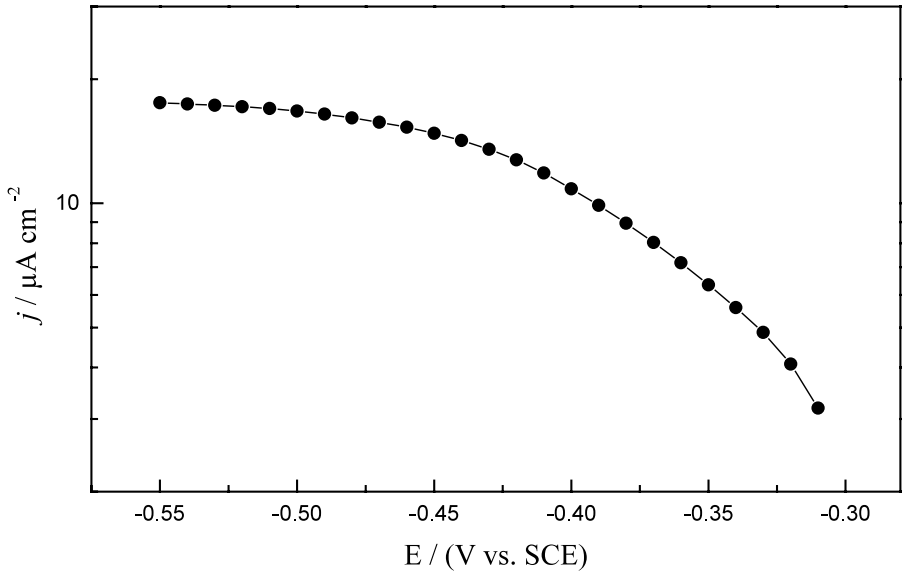

Fig. 2. Cathodic polarisation curve of aged aluminium brass in sterile culture media.

(SP). Even when in all the cases cultures were harvested after 25 h of growth, the catalase activity in the various cultures was found to be variable, as could be expected for an enzymatic activity whose expression depends on various different stimuli [15]. Besides this variability, it is important to note the contrast to the situation found in exponential phase (EP) cell-free cultures, where no activity could be detected (Table 1).

A typical polarisation curve registered on an aged electrode in aerated ASW is shown in Fig. 2 and will be used as a reference (control values). Polarisation results expressed as current density increments (%) with respect to control values are shown in Fig. 3. SP cell-free cultures induced current increments throughout the whole potential range tested, particularly in the limiting oxygen reduction region where the increment reached up to 35%. Currents in the activated region also increased in about 30% (Fig. 3), indicating that the electron transfer rate at the interface was enhanced. When measurements were performed with EP cell-free cultures in the absence of catalase (Table 1), current increments were only observed in the activated region, showing *no increment* in the limiting current (Fig. 3).

3.2. The influence of the composition of the surface layer on the catalase mechanism

As introduced above, the action of the catalase mechanism is dependent on both, the electrochemical generation of H_2O_2 during the oxygen reduction, and its accumulation at the interface [13]. As shown in Fig. 1, H_2O_2 is generated as an intermediate product which can be readily reduced to hydroxyl ions in a subsequent electrochemical step, or can be transferred to the solution by desorption. It is well established that oxides forming the passive film of copper and copper alloys play a key role in both, the hydrogen peroxide and in the oxygen reduction mechanisms [18,21–23]. Hydrogen peroxide reduction proceeds through the chemical oxidation of Cu_2O, to yield CuO. Cupric oxide is then electrochemically reduced to regenerate Cu_2O [21]. Cu_2O acts as a redox mediator allowing the fast electroreduction of H_2O_2. On a CuO surface on the other hand, the reduction of peroxide is inhibited and H_2O_2 is accumulated in the interface to finally desorb to the solution.

The production of H_2O_2 during oxygen reduction has been confirmed on aluminium brass [13]. Based on the assumption that, as in the case of copper surfaces, H_2O_2 reduction kinetics is determined by the surface film composition, experiments were devised to determine the influence of pre-conditioning treatments on the action of the catalase mechanism.

Polarisation curves were performed using pre-reduced and -oxidised surfaces and SP cell-free cultures in order to compare them with those in Fig. 3. Results are shown in Fig. 4. When pre-oxidised surfaces were used, current density increments in the diffusional region were considerably higher than those obtained with aged surfaces (Fig. 3). The oxygen reduction limiting current reached an almost 60% increment as compared to the corresponding control values (Fig. 4). On the other hand, when pre-reduced surfaces were used, current increments were completely absent (Fig. 4).

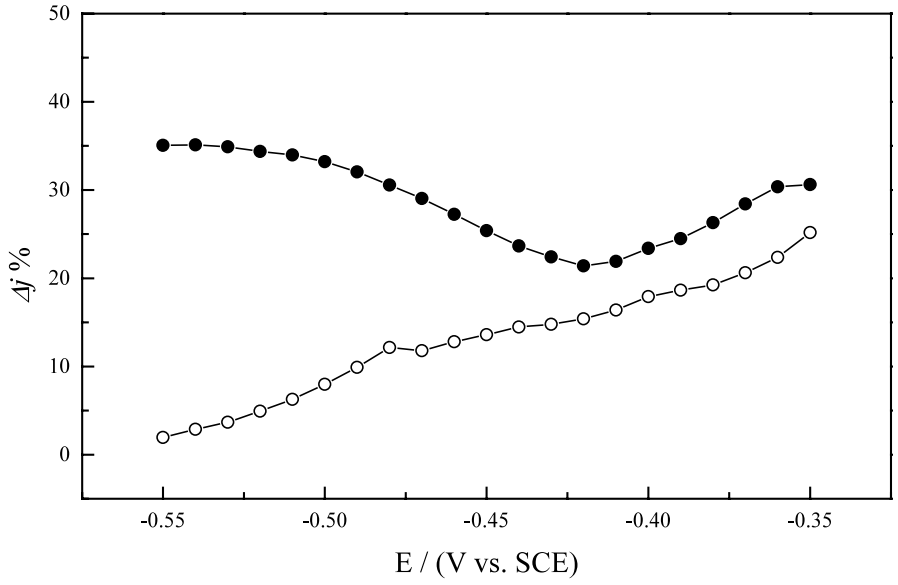

Fig. 3. Current density increments (Δj%) in cathodic polarisation curves of aged aluminium brass in stationary (●), and exponential (○) phase cell-free cultures of *Pseudomonas sp*. Results are expressed as percent of increment taking values in Fig. 2 as reference.

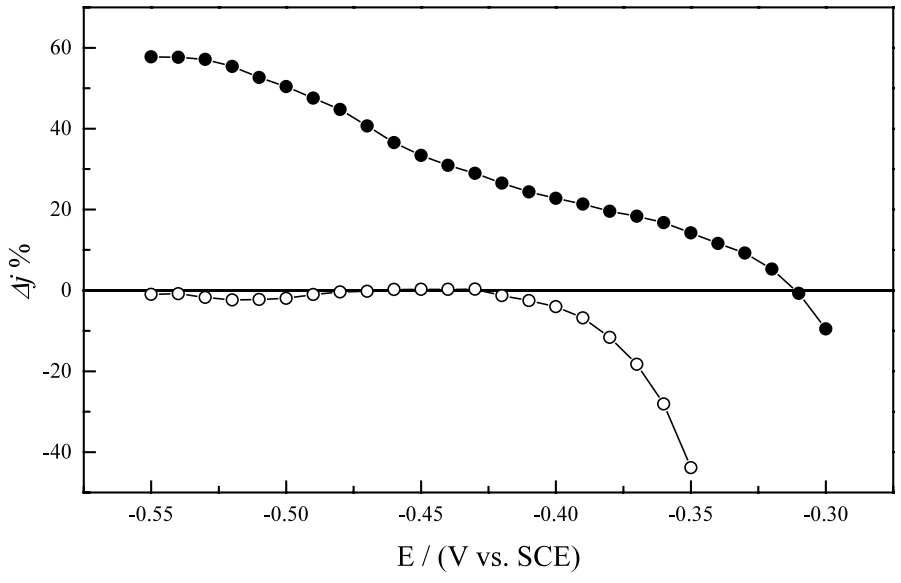

Fig. 4. Current density increments (Δj%) in cathodic polarisation curves of pre-oxidised (●), and pre-reduced (○) aluminium brass in stationary phase cell-free cultures of *Pseudomonas sp*. Results are expressed as percent of increments relative to values obtained in non-inoculated culture media.

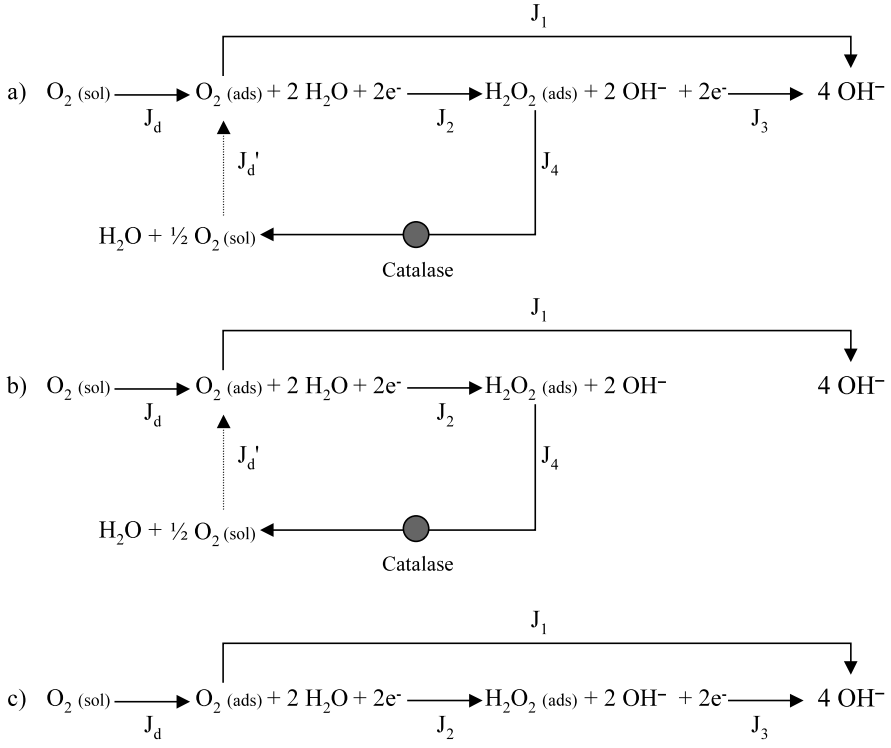

Fig. 5. Schematic representation of the oxygen reduction process on: (a) aged; (b) pre-oxidised; and (c) pre-reduced surfaces of aluminium brass. J_n represents the mass flux at step n. Species in solution (sol), or adsorbed to the surface (ads) are indicated.

4. Discussion

4.1. The mechanism

From the results presented in Table 1 and Fig. 3, it can be observed that current density increments were detected only in the presence of catalase. This is in agreement with previous findings, where it has been shown that catalytic activity can be suppressed by heating cell-free cultures containing catalase to an enzyme denaturing temperature (80 °C) [13], and thus provide additional support to the participation of this enzyme in the process.

The various degrees of current increments observed on the reduction currents in the presence of catalase on the different surfaces tested (Figs. 3 and 4) can be rationalised considering changes in the peroxide reduction versus desorption balance (k_{III} vs. k_{IV} in Fig. 1). This balance is dependent on the surface layer composition, which can be modified by electrochemical pretreatment of the surface. The changes introduced by pre-reduction, pre-oxidation, and ageing treatments will be analysed below in order to explain the experimental results.

Considering again the general scheme for the mechanism shown in Fig. 1, the overall current density in the absence of catalase can be expressed as:

$$j = 4FJ_1 + 2FJ_2 + 2FJ_3 \quad (2)$$

where J_n represents the flux at step n and F is the Faraday constant. Adsorbed oxygen and hydrogen peroxide masses can be balanced as $J_d = J_1 + J_2$ and $J_2 = J_3 + J_4$.

By substitution, Eq. (2) can be rewritten as:

$$j = 4FJ_d - 2FJ_4 \quad (3)$$

When the process is analysed on pre-reduced copper surfaces [18], a single four electron reduction wave is observed, indicating that the contribution of the desorption term ($-2FJ_4$ in Eq. (3)) is negligible. On pre-reduced copper–nickel surfaces [23] on the other hand, the reduction of oxygen proceeds through the exchange of less than four electrons ($n = 3.5 \pm 0.2$), indicating that the relevance of the bifurcation point in the reduction sequence (Fig. 1) directly depends on the composition of the metallic surface.

In the case of completely oxidised copper-based surfaces, hydrogen peroxide reduction would be inhibited [21] ($J_3 \rightarrow 0$). In such situation, Eqs. (2) and (3) should be rewritten as:

$$j = 4FJ_1 + 2FJ_2 \quad (2')$$

$$j = 2FJ_d + 2FJ_1 \quad (3')$$

On this basis, schematic representations of the oxygen reduction mechanism on aged, pre-oxidised and -reduced aluminium–brass surfaces in the presence of catalase are shown in Fig. 5a–c, respectively. The effect

of the presence of catalase on the oxygen reduction currents and thus on the reaction rate will be discussed independently on the following sections considering the expressions presented above.

4.1.1. Aged surfaces

As shown in Fig. 3, current density increments of nearly 35% were measured in the presence of catalase. This situation can be rationalised assuming a fast enzymatic decomposition of the adsorbed peroxide (see Fig. 5a).

Rewriting the corresponding mass balances for adsorbed oxygen and hydrogen peroxide, and substituting into Eq. (2), the current density can be expressed as:

$$j = 4FJ_d + 2FJ'_d \quad (4)$$

Comparing now Eqs. (3) and (4), the 35% increment in current would be attributed to the presence of additional oxygen on the surface, resulting from the decomposition of the surface peroxide by catalase. It has to be taken into account that the term J'_d contributes to the current with half of the oxygen concentration than J_d.

During the ageing process on copper and copper alloys, the formation of a Cu_2O layer is followed by the growth of a CuO layer on top of it [18]. The presence of both species has been detected on aged aluminum–brass using differential reflectance spectroscopy [24], where an incomplete coverage by the top CuO film could be observed. Taking into account that hydrogen peroxide reduction is highly inhibited on CuO surfaces, the progress of the oxygen reduction reaction on this Cu_2O/CuO patched surface could lead to variable amounts of peroxide on the surface at different potentials. Thus, H_2O_2 concentration at the interface, and hence the effect of catalase, would be determined by the relative coverage distribution of both oxides on the electrode surface.

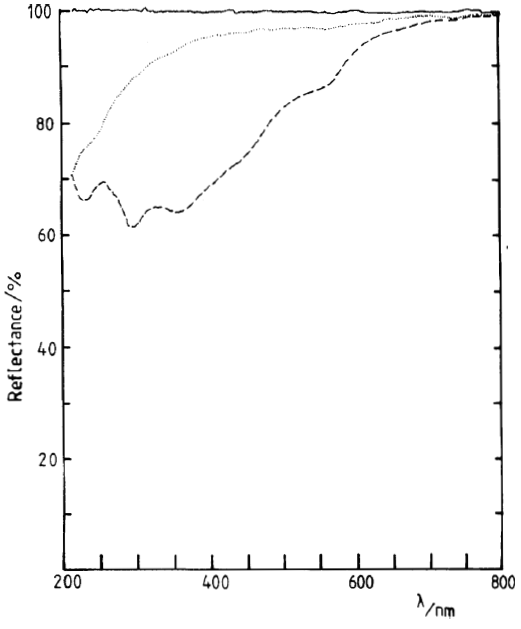

Fig. 6. Differential reflectance spectra of Cu(I) oxides in the absence (---), and Cu(II) compounds the presence of chloride (···). Reproduced with permission from Ref. [24].

4.1.2. Pre-oxidised surfaces

The pre-oxidation treatment applied to the electrode surface should lead to the formation of a Cu_2O film [26]. However, when chloride ions are present, as in the case here, Cu_2O dissolves and a film rich in Cu(II) compounds is preferentially formed [24]. Typical reflectance spectra of copper oxides grown in the presence and absence of chloride ions are shown in Fig. 6. On a surface mainly composed by Cu(II) species, H_2O_2 is poorly reduced favouring the accumulation of hydrogen peroxide on the surface [18] ($k_{III} \ll k_{IV}$, see Fig. 5b).

Assuming that J_3 is negligible when compared to J_2 and J_4, the corresponding mass balances would lead to the following expression for the current density in the presence of active catalase:

$$j = 2FJ_d + 2FJ_1 + 2FJ'_d \quad (5)$$

Clearly, a current density increment can be expected when Eq. (5) is compared to the current density in the absence of catalase on the same surface (see Eq. (3′)). Thus, the experimentally found increment of about 60% in current density shown in Fig. 4 could again be attributed to the reduction of the additional amounts of oxygen produced via the enzymatic decomposition of the hydrogen peroxide accumulated at the electrode surface.

As can be seen in Fig. 4, the increment measured on the current density depends on the electrode potential. This can be explained considering that one of the terms involved in Eq. (5) is potential-dependent (there is an electrochemical rate constant implied) while the others are merely diffusive. It is important to note that although J'_d contributes to the current with half of the oxygen concentration than J_d, as in the case of aged surfaces, with this treatment, and leaving aside the direct reduction via J_1, the electrons are recycled into a two-electron pathway inducing higher relative increments in the current density. Instead, in the case of aged electrodes (Fig. 5a) the additional O_2 incorporates into two consecutive two-electron steps, yielding a lower relative increase in current.

4.1.3. Pre-reduced surfaces

Using differential reflectance spectroscopy and ellipsometry it has been demonstrated that copper electrodes are covered by a Cu_2O submonolayer even after a pre-reduction treatment at -1.0 V in a solution deoxygenated by Ar bubbling [25]. The presence of this submonolayer of oxide was also shown on aluminium brass by ellipsometric measurements during cathodic polarisation [26].

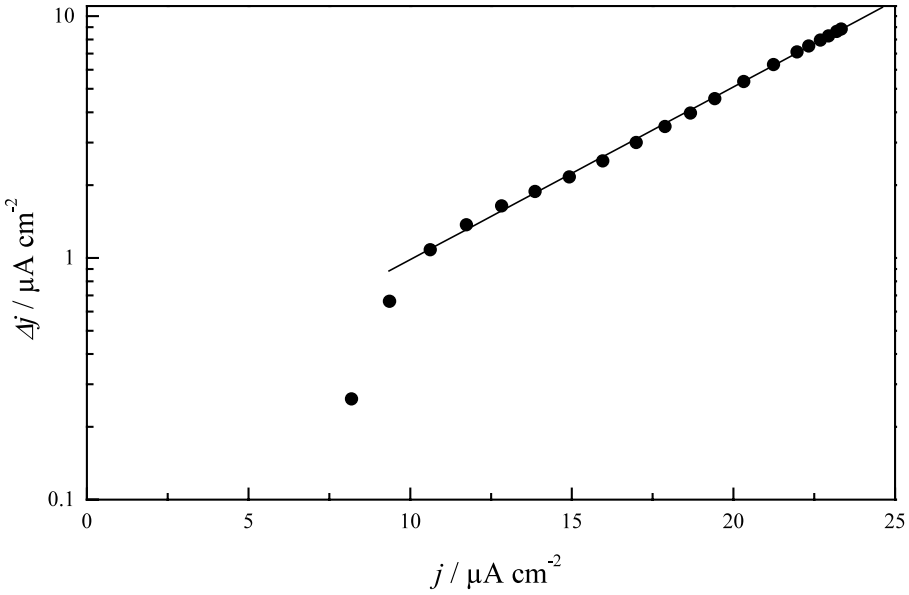

Fig. 7. Current density increments (Δj) as a function of the initial (control) current density (j) for every potential step in cathodic polarisation curves of pre-oxidised aluminium brass in stationary phase cell-free cultures of *Pseudomonas sp.* ($r = 0.9996$).

On this surface, hydrogen peroxide is probably reduced faster than it is desorbed [24]. It can be observed that in this case, when J_4 becomes negligible the enzyme action would be hindered and the current density would be represented by the expression proposed in Eq. (2). This would account for the complete lack of current increments in the presence of catalase in contact with pre-reduced surfaces.

4.2. Importance of the catalase mechanism in the influence of biofilms on corrosion

Key evidence relating the metabolism of aerobic biofilms on corrosion has been shown by several authors [6,7,9,12,13]. However, the detailed mechanism by which bacteria or their metabolites modify the electrochemical reactions at the interface remains obscure. To our knowledge, even when our results deal with planktonic cultures, the catalase mechanism is the first attempt to provide a mechanistic explanation for the depolarisation of the oxygen reduction reaction by an enzyme with a direct implication on the phenomenology of microbial corrosion in the presence of aerobic biofilms. The relative importance of the catalase mechanism in the overall microbial influence on corrosion process is difficult to determine, but it is worth noting that this mechanism is in agreement with most of the evidences so far reported, as summarised below.

(a) The depolarisation effect is reversible, the situation can be reverted by sodium azide [9]. This compound reacts reversibly with catalase yielding enzyme inhibition [12].
(b) The depolarisation effect disappears at temperatures higher than 40 °C [27], presumably due to the enzyme denaturalisation.
(c) A thick biofilm is necessary in order to produce the ennoblement and to restrict the dispersion of the biological catalyst to the liquid medium [12]. Catalase is mainly produced by stationary phase cells, and is probably induced at the bottom of thick mature biofilms where bacterial growth is limited by the exhaustion of nutrients [28].
(d) Mollica et al. [27] reported that an increment of cathodic currents was observed during 4–5 days after the onset of cathodic polarisation in the case of coupons on which biofilms have already been grown. The rate of change of cathodic currents was found to follow the relation: $\log i = \alpha t + \beta$, suggesting the participation of an autocatalytic mechanism which could be related to some extracellular bacterial metabolite [27]. The current density increments due to the action of catalase during polarisation curves follow the same relation as shown in Fig. 7. In this figure, current density increments for every potential step were plotted as a function of the initial (control) current density value to demonstrate the proportionality.
(e) Finally, a vegetative catalase protects biofilms against the penetration of H_2O_2 [29]. In addition, biofilms of *P. aeruginosa* showed an increased resistance to added H_2O_2, due to the expression of an inducible catalase (*katB*) [30]. Both, vegetative and inducible responses are expected to be active against electrochemically produced peroxide, and this can be the starting point of the autocatalytic

increment of cathodic currents on coupons covered by an aerobic biofilm.

5. Conclusions

Current density increments were measured in the presence of stationary phase cell-free cultures of *Pseudomonas sp*. and attributed to catalase activity. The catalytic effect of the enzyme was influenced by the surface treatments corresponding to various oxide layer compositions. The magnitude of the current increments can be interpreted by means of the stoichiometric calculations based on the catalase mechanism.

Results are in good agreement with most of the previous data concerning the ennoblement process during aerobic biofilms growth on metals. However, the relative incidence of the proposed mechanism on the overall microbial effects on corrosion process cannot be yet fully evaluated and needs further experimental work (already in progress).

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