Corrosion behaviour of AISI 304 stainless steel in presence of a biosurfactant produced by Pseudomonas fluorescens

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Abstract

Under certain conditions, some microorganisms are able to synthesize surface active compounds called biosurfactants (BS), which reduce the surface tension of water. BS characteristics depend on which microorganism produce them and therefore, on the microorganism culture conditions (temperature, pH, C, N-source, ...). Numerous applications are known for these biomolecules, such as cleaning, bioremediation, and their use as a detergent, or in cosmetic formulations.

Recently, a large amount of literature has been edited on the influence of BS on the interactions between pathogenic bacteria and inert surfaces. It has been shown that the modifications of surface properties by the adsorption of BS can reduce microbial adhesion.

Some other studies on chemical surfactants have shown that the adsorption of surface-active compounds plays a major role in corrosion; they are indeed used as an interesting corrosion inhibition tool. Therefore, it seems very interesting to study the impact of BS as environment-friendly (since biological and biodegradable) corrosion inhibitors.

In the present work, an attempt was made to study the corrosion behaviour of AISI 304 stainless steel in presence of BS produced by a Gram-negative bacteria, Pseudomonas fluorescens (Pf495). Corrosion tests were achieved on several surface oxidation states. The surface morphology of the corroded specimens was investigated using SEM.

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1. Introduction

Stainless steel is a material frequently used for its properties of resistance to corrosion in both the industrial domain and the maritime field. Indeed, in contact with the air, the surface is quickly covered with a chromium and iron oxide layer, which increases the resistance to corrosion. The quality and the evolution of this protective film depend not only on the environmental conditions close to the metallic surface but also and mainly on the chromium content of the alloy.

AISI 304 stainless steel, whose chromium content is equal to 18%, has been chosen in this study because of its frequent use and its relatively weak resistance to corrosion (in comparison with the other stainless steels), allowing to assess more easily the influence of the environment on corrosion.

In order to reinforce the resistance to corrosion of stainless steels, a surface conditioning can be achieved. Some authors [1,2] showed that the adsorption of a chemical surfactant is a good way to inhibit corrosion. However, very few studies have been devoted to biological surfactants considered as potential corrosion inhibitors. The aim of this paper was to analyse and predict the hypothetical inhibition of stainless steel corrosion by biosurfactants (BS) produced by the bacterium P. fluorescens.

In a previous study [3], authors using X-ray photoelectron spectroscopy (XPS spectra obtained on a VG Escalab MKII spectrometer equipped with an Al Kα X-ray source) had shown an irregular thick layer adsorbed on stainless steel surfaces conditioned by a biosurfactant produced by Pseudomonas fluorescens. These analyses [3] had revealed that the adsorption of these BS leads to a reduction of iron oxide formation on the metallic surface and to an enrichment in chromium oxide and hydroxide within the passive layer. So, the authors suggested the existence of protective properties of biological surfactants against corrosion.

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The biosurfactant used in this work is the same surface-active compound that was used by Meylheuc et al. [3–5]. The efficiency of this corrosion inhibitor is estimated by several laboratory tests.

Pitting corrosion is one of the typical kinds of corrosion of stainless steels. It occurs particularly where the metallic surface presents defects, irregularities, heterogeneities, and is accelerated in presence of chlorides. The resistance to pitting corrosion of these alloys is classically determined by pitting potential measurements; a galvanostatic technique has been used in this work.

The different environments that have been tested are:

1. Air: its action, which promotes the oxide formation, is well known. Two surface oxidation states are studied. This choice is based upon Le Bozec’s results [6]. The first state is obtained after polishing; in these conditions, [6] observed on AISI 316L a ≈2 nm thick homogeneous layer composed of oxides (FeO and Fe2O3) and (Cr2O3). The second is obtained after a 5 days ageing in contact with the ambient air, at the room temperature. Other XPS analysis [7] have shown that during the ageing of the passive film, the oxide layer evolves towards a bilayer structure: inner oxides film reached in Cr3+ and outer oxides film reached in Fe3+.

2. Sterile deionised water, with or without biosurfactants: surfaces are conditioned by immersion during 20h. The adsorption procedure was realised by sedimentation of the biosurfactant solution on the stainless steel surface [3,4].

The electrochemical tests of determination of the pitting potential are achieved in sterile deionised water containing NaCl 0.15M.

2. Experimental methods

2.1. Biosurfactants

Biosurfactants were produced on a solid medium, as previously described [4]. Briefly, plates of King’s B (KB) agar medium [8] were densely inoculated with two loops of a suspension of P. fluorescens 495 (obtained from C. Nguyen-The, INRA Montfavet, France). The inoculated plates were incubated during 4 days at 22 °C. The bacterial lawns were scraped from the agar and the cells were resuspended in sterile, deionised water and dispersed, using a vortex mixer operated at maximum speed. The supernatant containing the biosurfactant was separated from the cells by centrifuging at 18,000 × g for 30min. The supernatant was then filtered through a 0.22-µm pore-size filter and stored at 4 °C.

The ionic charge of the biosurfactant was determined using the agar double diffusion technique [10,11]. As previously described [4], two regularly spaced rows of wells were made in an agar of low hardness (1% agar). Wells of one row were filled with the biosurfactant solution and wells of the other were filled with a pure compound of known ionic charge.

The anionic substance chosen was sodium dodecyl sulfate (SDS) 20 mM and the cationic one was barium chloride, 50 mM. The appearance of precipitation lines between the wells, indicative of the ionic character of the biosurfactant, was monitored over a 48-h period at ambient temperature.

2.2. Materials

The material used for this study was a commercial AISI 304 stainless steel; the chemical composition of which is given in Table 1.

Several pre-treatments of the electrode surface were tested:

(a) “Polished surface”: specimens were prepared by mechanical polishing with SiC paper up to 1200 grit, washed with sterile deionised water, dried in the air and used after 15 min.

(b) “Aged surface”: specimens were prepared as in the “polished surface” pre-treatment but used after a 5 days ageing in contact with the air.

(c) “Conditioned surface”: polished or aged surfaces were immersed during 20h in the solution of biosurfactants (BS495) containing surface-active compounds produced by P. fluorescens 495. The test area was exposed towards the top of the solution, so that the BS sedimentation and adsorption could occur. Then the samples were rinsed with sterile deionised water.

(d) Conditioned surface in sterile deionised water (W): specimens were immersed during 20h in sterile deionised water.

The exposed area of the metallic specimens was of 6.25 cm². Prior to the experiment, the sample was rinsed with sterile deionised water.

2.3. Electrochemical measurements

All experiments were carried out without any agitation of the electrolyte, and the concentration in dissolved oxygen results of the atmospheric pressure. An experiment was realised in a deaerated solution to study the influence of the dissolved oxygen. The solution was deaerated by nitrogen bubbling during one hour. In order to work in the same condition as in the other experiments (no agitation) the bubbling was stopped and a nitrogen atmosphere was maintained above the solution during all the experiment.

During the conditioning procedure of stainless steel surfaces (i.e. the immersion in BS495 solution), the free corrosion potentials were recorded.

Table 1. Chemical composition (% weight) of the alloy AISI 304

<table>
<thead>
<tr>
<th>Element (%)</th>
<th>AISI 304</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>18.27</td>
</tr>
<tr>
<td>Ni</td>
<td>8.66</td>
</tr>
<tr>
<td>C</td>
<td>0.047</td>
</tr>
<tr>
<td>Mn</td>
<td>1.19</td>
</tr>
<tr>
<td>N</td>
<td>0.078</td>
</tr>
<tr>
<td>S</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

The material used for this study was a commercial AISI 304 stainless steel; the chemical composition of which is given in Table 1.
Polarization was applied with a potentiostat linked to a classical three electrodes electrochemical cell. All the potentials were measured and reported vs. the SCE. The working electrode was the AISI 304 sample.

The galvanostatic anodic polarization and cathodic polarization methods were carried out in NaCl 0.15 M solution. The galvanostatic anodic polarization method used in this work was described in detail by Frangini et al. [12,13]. A typical galvanostatic curve obtained during a chronopotentiometric experiment is presented in Fig. 1. A current density of 10 µA/cm² was imposed during 15 min to the sample. The measurement period was 0.1 s. The maximum value reached by the potential is the pitting potential ($E_p$).

In the beginning, the value of the potential increases regularly and very quickly until it reaches a maximum value (pit nucleation potential, “$E_p$”). Beyond this maximum value, instabilities appear, indicating the formation of pits. Then the amplitude of these instabilities weakens and the values of the potential decrease until pits are repassivated.

The cathodic polarization curves were recorded by linear scan voltammetry (5 mV/s). The potential sweep started from 0 V/SCE (near the free potential) to $-1.5$ V/SCE. According to the literature, in this range of potentials, the reduction of dissolved oxygen can be observed; a peak was located round $-0.6$ V/SCE. The reduction of iron oxide took place simultaneously, but this reaction was often concealed by the reduction of dissolved oxygen [6,7].

2.4. Observations

Scanning electron microscopy (SEM) was used to observe the formation of the pits.

3. Results

3.1. Biosurfactant production and characteristics

In our experimental conditions, the solution of biosurfactant produced by *P. fluorescens* 495 lowered the surface tension of water to 27.4 mN m⁻¹. It was checked that the supernatant, non-inoculated of scraped Petri dishes of KBA (King B Agar), had its surface tension enclosed to that of water, thus indicating the absence of measurable surfactant activity. Agar double diffusion tests revealed the appearance of precipitation lines between the biosurfactant produced by *P. fluorescens* 495 and the cationic compound selected (barium chloride), while no lines had formed between the biosurfactant and the anionic compound (SDS). Under the prevailing experimental conditions, this very simple test demonstrates the anionic character of the biosurfactant produced.

3.2. Galvanostatic test

The determination of the pitting potential ($E_p$) corresponding to pre-treatments of the electrode surface was realised by the galvanostatic test.

Before this test, the samples were immersed in NaCl 0.15 M during 15 min, so that the free potential could reach a stationary value.

The peak values of the potential were noted and reported in Fig. 2. In order to control the reproducibility, several tests have been carried out.
3.3. Oxidation due to air

The influence of air [7,14] on the formation of the passive layer of this alloy was confirmed and the values of the pitting potential were used as reference to classify all the data obtained with the same method. The \( E_p \) value of the samples was superior in the case of aged surface (390 ± 10 mV/SCE) compared to a polished surface (260 ± 30 mV/SCE) (Fig. 2).

3.4. Biosurfactant

The solution containing the Pf495-biosurfactant (BS495) was sterile deionised water, it appeared important to test first the reactivity of the surfaces conditioned by this water; then some identical tests have been achieved after adding the BS495.

3.4.1. Sterile deionised water

The pitting potential obtained for the aged surface (520 ± 15 mV/SCE) was also superior to the one obtained for the polished surface (445 ± 10 mV/SCE). The immersion during 20 h in sterile deionised water had promoted the surface oxidation [15]. The immersion in sterile water had promoted the shift of \( E_p \) toward more positive values with a gap (≈150 mV) (Fig. 2).

These different oxidization states were confirmed by measuring the evolution of the free potential (\( E_f \)) during the immersion (Fig. 3). After 20 h, the free potential had shifted toward more positive values (≈150 mV), and the final value of \( E_f \) was higher when the surface was aged.

3.4.2. BS produced by P. fluorescens 495 (Pf495)

In presence of the adsorbed biosurfactant BS495, the highest pitting potential was not the aged surface \( E_p \) anymore (410 ± 25 mV/SCE) but the polished surface \( E_p \) (435 ± 5 mV/SCE). The action of the biosurfactant as a corrosion inhibitor seems to be more efficient if its adsorption occurs on a more active surface, but the gap of values was weak. It could be explained by the results presented in Fig. 4.

At the beginning of the immersion, a significant gap of free potentials appeared but quickly the \( E_f \) values of the polished surface samples grew closer to the \( E_f \) values of the aged surface samples. The evolution of the free potential in the BS495 solution was weak (≈25 mV) whereas it was about 100 mV in the sterile deionised water (Fig. 3).

After 20 h, the free potential values were very close. It indicated that the interface metal/solution was in a quite similar state, that is why \( E_p \) was similar whatever the pre-treatment. The presence of biosurfactant on the surface inhibits the stainless steel oxidation.

3.5. Potentiodynamic tests

These tests characterizing the reduction of the oxygen dissolved in solution and the reduction of iron oxides were achieved in NaCl 0.15 M.

As it is classically reported in the literature [6,7,16], a wave was noticed at the cathodic potential \(-0.6\) V/SCE. This wave corresponds mainly to the consumption of oxygen dissolved in solution. The diffusion current of the oxygen was then masked at potentials lower than \(-1\) V/SCE; at these potentials, the current density results mainly from the water reduction with dihydrogen formation.

To make sure that the cathodic current evolution noted between \(-0.4\) and \(-0.9\) V/SCE was mainly due to the oxygen reduction, two experiments were carried out.
The first one was realised in NaCl 0.15 M at atmospheric pressure. In this experiment some oxygen was dissolved in the solution. This oxygen was eliminated of the electrolyte by nitrogen bubbling in the second experiment.

Results are given in Fig. 5. Between $-0.4$ and $-0.9$ V/SCE, the current density of the curve obtained with the deaerated solution (Fig. 5b) was weaker than the one obtained with naturally aerated solution (Fig. 5a). The current densities were similar only during dihydrogen formation for potentials lower than $\sim -1$ V/SCE.

Consequently, current densities measured between $-0.4$ and $-0.9$ V/SCE can be considered mainly as oxygen reduction.

Curves of potentiodynamic tests obtained on unconditioned surfaces (I) or surfaces conditioned (II) in sterile deionised water (W) are shown in Fig. 6. The curves (a) correspond to the aged surfaces and the curves (b) correspond to the polished surfaces. Whatever the conditions, the results were similar. These different treatments do not have an effect on the reduction of dissolved oxygen.

When the stainless steel surface was covered by the Pf495-biosurfactant, the wave of the reduction of oxygen did not appear anymore, as it is clearly visible in Fig. 7; a slight shoulder appeared but it was difficult to assign it to the polished or aged surface. The conditioning could provoke an irregular thickness of the adsorbed BS495 film and so the barrier effect on the oxygen diffusion could be different from one test to another. The adsorbed BS495 film is an efficient barrier of diffusion for the dissolved oxygen.

This effect of the biosurfactant adsorption on the surface was still effective when the samples were rinsed with sterile deionised water before being tested, as explained in the literature [3].

4. Discussion

In different studies, XPS surface analysis has shown that the composition and the structure of the oxides layer formed on stainless steel surfaces depend on the environment [3,6,7,14,15]. Thus, after polishing, a very thin layer of iron and chromium oxides appears with a homogeneous distribution. Time passing by, the layer grows and its composition evolves. A stratification appears, the contents in chromium and iron oxides varying according to the environment stainless steels are exposed to.

According to these authors, the surface preparations that have been studied in this work have a different nature: a polished surface covered by a thin and homogeneous layer, whereas an aged surface covered by an oxide-stratified layer. This layer evolves with the solution in which the samples are immersed.

Different oxide layers lead to different resistances to corrosion. This effect can be studied by pitting potential measurements.

The polished surface has a thin, less protective, oxide layer and the pitting potential is weak ($E_p = 260 \pm 30$ mV/SCE). The surface conditioning by action of dioxygen shifted this potential toward more positive values (aged surface, $E_p = 390 \pm 10$ mV/SCE).

The action of dissolved oxygen in sterile deionised water is still more efficient, because the potential values are more positive: $E_p = 445 \pm 10$ mV/SCE for the polished surface and $E_p = 520 \pm 15$ mV/SCE for the aged surface. These different values show that these various working conditions produce different surface states.

A previous study [3] has shown by XPS surface analysis that the adsorption of Pf495-biosurfactant on AISI 304 inhibits the formation of the oxide layer. The $E_p$ values noted in this work for the samples whose surface has been conditioned by the biosurfactant were expected to be closer to the $E_p$ values of the polished surface samples than to the $E_p$ values of the aged surface samples. However, $E_p$ values were a little more positive for the polished surface ($E_p = 435 \pm 5$ mV/SCE) than for the aged surface ($E_p = 410 \pm 25$ mV/SCE). These results are contrary to the other tests where $E_p$ was always higher for aged

![Fig. 6. Potentiodynamic tests in NaCl 0.15 M, from 0 to $-1.2$ V/SCE. Scanning rate: 5 mV/s. Before testing: (I) unconditioned surface; (II) sample immersed during 20 h in sterile deionised water (W); (a) aged surface; (b) polished surface.](image1)

![Fig. 7. Potentiodynamic tests in NaCl 0.15 M, from 0 to $-1.2$ V/SCE, scanning rate: 5 mV/s. Samples conditioned by the Pf495-biosurfactant before testing: (a) aged surface; (b) polished surface.](image2)
surfaces. This shows the efficiency of the biosurfactant, which acts as a quickly-appearing barrier to the chloride diffusion.

The electrochemical behaviour of stainless steels has been studied by comparing their pitting corrosion resistances. Other studies [6,7,16] show that the rate of the cathodic reaction (water and dissolved oxygen reductions) is an important parameter to understand the corrosion behaviour of stainless steels.

The value of the current density of the cathodic reactions (oxygen reduction occurring near $-0.6 \text{ V/SCE}$, water reduction round $-1 \text{ V/SCE}$) influences the value of the free corrosion potential. If the anodic metal dissolution is considered as constant, the weaker the oxygen reduction will be, the more negative the free corrosion potential will be.

Thus the value of the free corrosion potential depends not only on the surface oxidation state, but also on the cathodic reaction of oxygen reduction.

This explains the results observed in this work. The highest free corrosion potentials are obtained for strongly-oxidized surfaces (aged during 5 days in contact with the air followed by an immersion of 20 h in a sterile deionised water solution). The lowest potentials are noted for samples whose surface has been conditioned by BS495. The adsorption of biosurfactant inhibits the oxide formation on the surface of stainless steels.

Therefore, to reach the pitting potential from the free potential, it was necessary to impose to the samples conditioned by the biosurfactant an energy superior to the one necessary for aged surfaces immersed in sterile deionised water before the pitting test.

If the potential of the sample conditioned by the biosurfactant is superior to its pitting potential, then the corrosion will appear very quickly, because pits are initiated on a surface which is not protected by an oxide layer. The oxide layer does not have the time to grow before the adsorption of the biosurfactant.

As shown on the SEM observations (Fig. 8), pits initiated in the NaCl 0.15 M solution are large and not very deep for the samples conditioned by biosurfactant, while pits have a low diameter and grow in depth on aged surfaces immersed in sterile deionised water during 20 h before pitting test.

For samples conditioned by the biosurfactant, the chlorides will react easily with the metallic surface. The protective oxide layer does not have the time to develop because the adsorption of the biosurfactant occurs very quickly. In these conditions the corrosion can propagate more easily on the surface.

When the sample is immersed in sterile deionised water without the biosurfactant BS495, the oxide layer is more compact, the number of defects decreases, which leads to a lower density of pits and to a higher cathodic surface/anodic surface ratio. This could explain the depth of pits.

Thus, if the potential of the samples conditioned by the biosurfactant is superior to their pitting potential, pits will form and develop in width, disturbing the biosurfactant adsorption, and so the protective effect against corrosion will not be efficient.

5. Conclusion

The biosurfactant produced by *P. fluorescens* 495 and the oxide layer both act as barriers to the diffusion of compounds such as dissolved oxygen or chlorides.

The diffusion of dissolved oxygen has been studied by observing the cathodic reaction between 0 and $-1.2 \text{ V/SCE}$,
while the diffusion and the effects of chlorides have been evaluated by pitting potential measurements.

The biosurfactant adsorption occurs faster (electrostatic interactions) than the oxide formation (atomic diffusion). It is the reason why the adsorption of these surface-active compounds slows down the diffusion of dissolved oxygen. This is favourable in the aggressive solutions where the oxygen is the main oxidizing agent, but it is harmful to the formation of the protective oxide layer.

In the more aggressive solutions (NaCl) the biosurfactant layer, like the oxide layer, slows down the diffusion of Cl\(^{-}\). However, if the chloride ions come close to the metallic surface conditioned by the biosurfactant BS495, the corrosion will grow quickly because the protective oxide layer have no time to nucleate before the adsorption of the biosurfactant. In these conditions, the surface is weakly aged, the pits develop in width, the corrosion is quick and the biosurfactant adsorption is strongly disturbed.

So the biosurfactant will delay the corrosion of stainless steels, especially if the electrolyte is not too aggressive. In aggressive solutions, when the corrosion appears, it is increased in the presence of BS495.

To improve the action of the biosurfactant as a corrosion inhibitor, it is necessary to have a prepassivated surface before the biosurfactant adsorption. It is the reason why future works will focus on the effect of different surface preparations prior to the biosurfactant adsorption.

References