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MICROBIOLOGICAL CHARACTERIZATION OF *Erwinia Genus* ON THE SURFACE OF STAINLESS STEEL AISI 316L

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ABSTRACT

In this article, the behaviour of acid-producing bacterium *Erwinia genus* is characterized on the surface of stainless steel AISI 316L. Microbiological characterization was carried out by macroscopic and microscopic morphological tests and biochemical tests; also the growth kinetics was established on the steel surface through periodic reviews and Petro Hausser chamber and the electrochemical impedance diagrams characterization and Tafel polarization curves. The results showed the growth of colonies in the surface material slow and stable as to the steel surface increased corrosion current influenced by bacterial growth is observed.

Key words: *Erwinia genus*, Corrosion, Stainless Steel, Microbiological.

INTRODUCTION

Among stainless steels, those with austenitic microstructure are the most common in the market due to the extraordinary mechanical corrosion resistance, excellent appearance, and excellent ductility, good weldability properties, machining, cutting, bending and folding. Stainless steels containing alloying elements Cr (16-26 mass %) and Ni (6-22 mass %)[1,2], which exert a significant synergistic effect, Chromium provides the stainlessness and nickel favors austenitic its microstructure, They include lower percentages of other alloying elements such as Mn, Si, C, Mo, N, Ti, Nb important materials that provide the mechanical properties and resistance to corrosion characteristics[3-5].

However, oxidation of these steels form an invisible and adherent thick layer covering the surface of the material[6,7]. Likewise, the presence of other alloying elements makes other phases contributing characteristics alloy affecting corrosion resistance phrases as carbides, nitrides, carbonitrides, where the formation of sigma phase (brittle and hard

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intermediate phase of tetragonal structure) in the grain boundaries gives brittleness and cause sensitization to intergranular corrosion in some means[8,9].

One of the problems affecting industries using austenitic steels is the metabolic activity of microorganisms on metallic materials generating an impact of electrochemical potential over a wide range of industrial environments compromising the integrity of the materials used in facilities and processes[10]. The acid producing bacteria contribute to corrosion processes in metals by dissolution under conditions created acid, these species are registered under reducing conditions within biofilms and sometimes their activity is not perceived as lateral erosion and that are located in biomass-metal interface and it produces a biofilm as a defense mechanism against external agents and facilitates the accumulation of organic and inorganic substances for bacterial growth[11].

In this article, the study of macroscopic, microscopic morphological characters, growth and affected by acid producing bacteria in austenitic steels was carried out.

EXPERIMENTAL

To determine the effect of acid producing bacteria samples are prepared stainless steel AISI 316 L. The chemical composition of base material, obtained by analysis of X-ray fluorescence is presented in Table 1.

Table 1: Chemical composition of steel AISI 316L

Percentage (%)	Compounds
18.5	Cr
13.4	Ni
3.8	Mo
1.9	Mn
0.75	Si
0.03	S
0.03	C

For preparation of the specimens were polished with sandpaper different from the number 80 to 1200 until leaving with a mirror-like luster, bacterial strains were extracted from a corrosive metal, which were grown by bacteriological to 37°C, pH 7.5 and in a culture medium with the composition as evidenced in Table 2[9,10].

For the characterization of bacterial Gram's method is used for observation of microscopic morphological and gender of the strain was confirmed by biochemical tests: oxidase, carbohydrate fermentation, nitrate reduction, catalase and through Bergeys Manual.

Each of the strains was incubated in samples of steel with temperature fluctuations of 30 to 40°C, pH 7.9 and 7.0 in a culture medium Table 2.

Table 2: Culture medium composition

Percentage	Compounds
20 g	Pancreatic digest of casein
9 g	Phenol red
10 g	Dextrose
20 g	Agar
2 g	Sodium thioglycolate
2 mg	Methylene blue
1000 mL	Distilled water q.s.

The culture medium was prepared according to the NACE TMO 194-2004 Standard, wherein the culture medium was distributed in Petri dishes, which were subjected to a nitrogen stream displacing oxygen to assimilate the anaerobic environment. Subsequently, the samples of AISI 316L were subjected to exposure of the bacterial strain for 480 hrs incubation period at a temperature range of 30 to 40°C, pH 7.9 and 7.0 to a culture medium.

For biochemical tests colonies, they were identified using conventional biochemical tests:

Catalase test: It was noted with inoculum from the agar medium for the test was performed by a drop of a glass slide H_2O_2.

Oxidase test: Test strips Bactident oxidase Merck was used, respectively.

Carbohydrate fermentation: To determine the fermentation of carbohydrates phenol red was used separately for each of the carbohydrates; then sterilized medium 10 g/L were added each carbohydrate.

Urease activity test was prepared a Christensen base was performed the autoclaving process where 5 mL of a solution of 40% urea was added. To study the kinetics of growth of

acid-producing bacteria, monitoring for a period of 480 hours of incubation where regular measurements were taken every 24 hours to identify the phases of bacterial growth, by a direct method performed the cell dry weight and the counting the number of cells[12].

To count the number of cells was performed by using the Petroff-Hausser, camera has a grid is divided into 25 fields recorded on the bottom of the chamber; each of the fields is subdivided in a grid of 16 squares[13,14]. Phase contrast microscope with a 40X objective was used for counting bacteria in only 5 of the 25 fields[15]. It can calculate the number of microorganisms in a sample, from the volume of the chamber and sample dilutions necessary. The equation (1) for this determination is[16]:

$$\frac{\text{Number of bacteria}}{\text{mL}} = \frac{\sum \text{Cells counted in the fields}}{4} \times \frac{1}{\text{Dilution}} \times 10^6 \qquad \ldots(1)$$

Electrochemical techniques were performed according to ASTM G1 and G3 regarding electrochemical tests. All the electrochemical tests were performed with parameters of time 306, 408 and 528 hours incubation of acid-producing bacteria in order to establish the bacterial growth in each sample and the wear caused to the steel surface[17].

The electrochemical tests were performed in a Gamry model PCI 4, at room temperature. The immersion of the samples were made immersing the samples and it is performed in a solution containing 3.5 wt% sodium chloride analytical. The type of reference electrode used in all tests was an electrode of Ag/AgCl of type Analytical Electrochemistry BAS (MF- 2052 RE -5B), which has a potential of + 0,194 V with respect to reference electrode hydrogen room temperature. All potentials are referred to the potential of the electrode Ag/AgCl[18,19]. In potentiostatic and impedance spectroscopy tests, a platinum counter electrode was used. The Nyquist diagrams were obtained with frequency sweeps between 100 kHz and 0.001 Hz using sinusoidal signal amplitude of 10 mV and exposed area of 1 cm^2. Tafel plots were obtained at a scan rate of 0.5 mV/s in a range of voltages from -0.25 V to 1V.

RESULTS AND DISCUSSION

Bacterial characterization

Macroscopic morphological characterization

As macroscopic morphological characters colonies are characterized by white, circular shape, smooth surface, slightly elevated, margin entire, creamy consistency. In each of the colonies acid formation is evidenced of Fig. 1 (a) and (b) evidence.

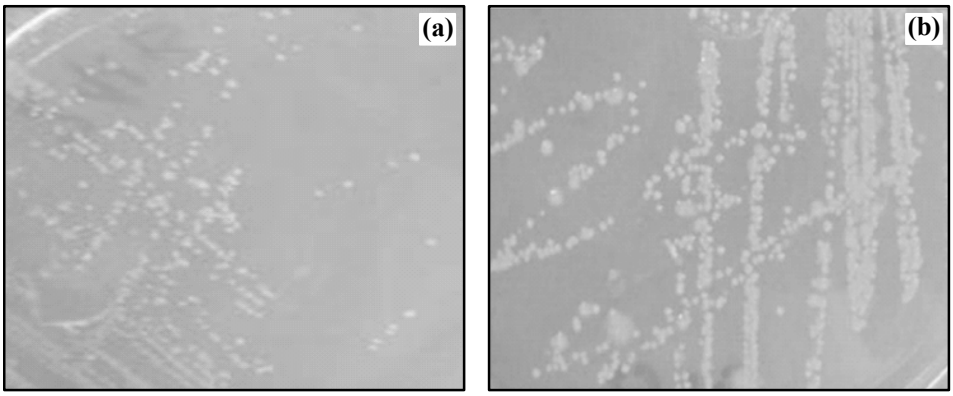

Fig. 1: (a) and (b) Morphological characteristics colonies acid producing

Bacteria bacterial cells show staining gram negative, morphology bacillary straight type, short and small. The bacterial disposition was individual, in pairs or aggregated as seen in Fig. 2 (a) and (b).

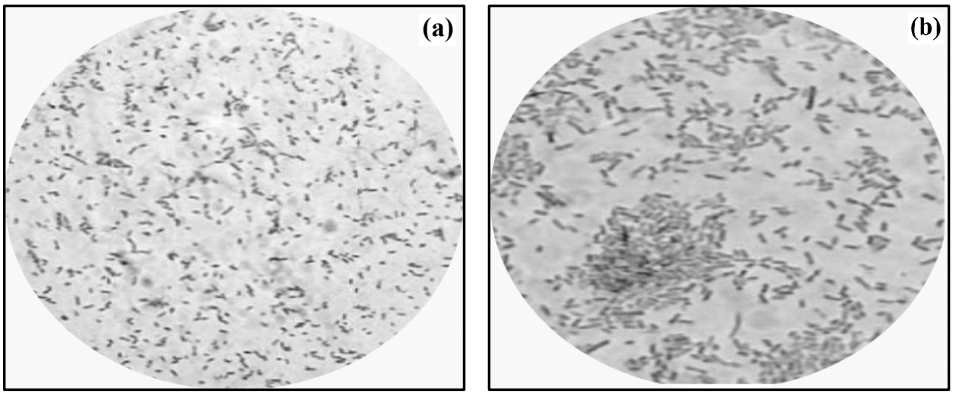

Fig. 2: (a) and b) Bacterial cells with 100X

Biochemical characteristics

Catalase test

For this test, the result is positive because the presence of bubbles in the culture medium. The catalase enzyme is a haemoprotein with four atoms of iron in reduced state. The H_2O_2 emerges as the final products of oxidative metabolism or aerobic in carbohydrates.

Oxidase test

According to the test with strips Bactident of the Oxidase, TM was negative due to

metabolism of the bacteria because it needs oxygen for their respiratory processes.

Carbohydrate fermentation test

The fermentation of carbohydrates as mannitol, glucose, sucrose and sorbitol was positive and this is observed as colouring medium changes from red to yellow by the positive reaction of the fermentation. The breakdown of carbohydrates is important in determining the fermentative bacteria are like family Enterobacteriaceae[20].

Given morphological characters, biochemical tests such as lactose negative, absence of urease activity and no phenylalanine you can identify, which colonies belong to the family Enterobacteriaceae and the *Erwinia genus*[21]. As for the role of these bacteria in bacterial corrosion, it is inferred that the acid produced by the metabolic activities reacts with carbon caused wear on the surface of the material, which is used by the sulphate-reducing bacteria to create processes pitting corrosion.

Growth kinetics

In Fig. 3, we can see bacterial growth on the surface of steel AISI 316 L of the *Erwinia genus*. The growth curve is homogeneous and the adaptation phase occurs from 96 to 264 hrs where cell growth begins, a layer of whitish cream colour is evident in the steel surface and growth is slow and steady. Growth is slow of the *Erwinia* genus can infer that because the nickel acts as a microbial agent; lactic acids also react stably with carbon levels AISI 316 L making it generates a compact biofilm[22].

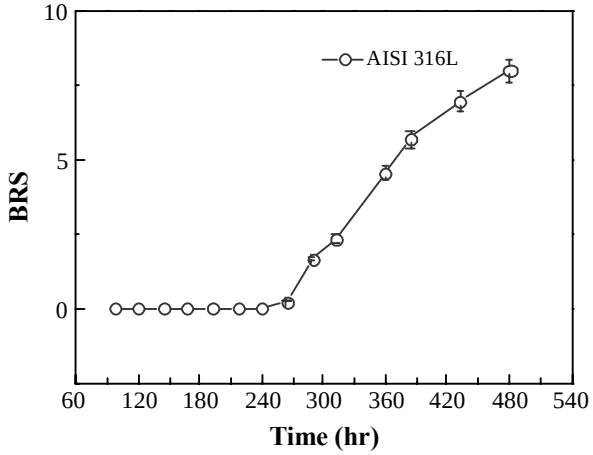

Fig. 3: Growth kinetics *Erwinia genus* steel AISI 316 L

Electrochemical characterization

The corrosion properties of AISI 316L steel after subjecting them to the bacterial strain during the time range were evaluated by Tafel polarization curves and electrochemical impedance spectroscopy (EIS).

In Table 3, the results of Tafel polarization curves, where the curves after the test performed show a range of values for the corrosion potential of -0.26 to ranging from -0.67 V vs Ag/AgCl are observed, all the curves show a behaviour in the anodic zone anodic dissolution (Fig. 4), to calculate the exchange current density was determined that the process is under anodic control, when analysing the data obtained experimentally. It can be determined that the process is under anodic control, and it generates the slowest reaction is the passage of the electron to the metal surface and the metal ion to the surface this is a simple way to describe it. This process also plays an important role that the film formed in the surface a hydroxide makes this process more complex[23]. So it can be said that the process of the surface to a greater extent reflected in the results[11]. In the kinetic process the corrosion currents are very susceptible to changes in the surface[12].

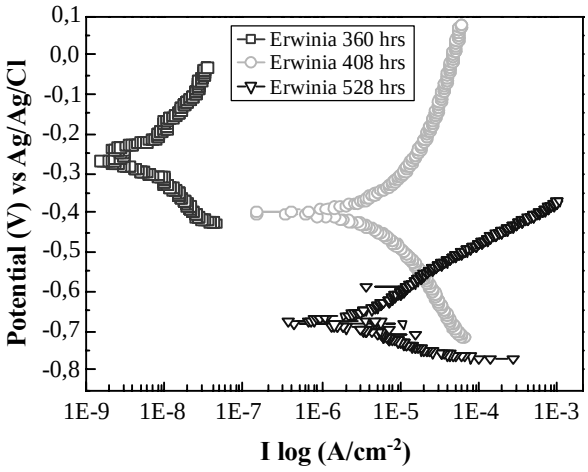

Fig. 4: Cathodic polarization curves corresponding to bacteria *Erwinia genus* evaluated at different times of evaluation

The lowest values of corrosion were obtained at 360 hours, then the increase was high and stabilized between 408 and 528 hrs, with respect to the anodic areas, it can be observed that for a time evaluation of 360 hrs is generated a passivation zone to -0.23 V vs Ag/AgCl. This area is generated due to the chromium oxide layer that is generated by the action of bacteria. After this potential a small increase in current is generated, and the protective layer is

gradually dissolved with no presence of a passive layer because there is greater bacterial growth, no passive layer by bacterial growth, which influences the consumption of the passive layer, by increasing the time of bacterial growth, is generated increased corrosion current and an increase in corrosion rate influenced by bacterial growth[14].

In Table 3, the values generated by the polarization curves are obtained. It is observed that as there is an increase in evaluation time less corrosion potential is generated, with movement towards the cathode regions, with respect to current and corrosion rate. It is established that there is an increase of these parameters as indicated above because there is an active corrosion after 360 hours the that compromise chromium oxide layer. It is generating substantial increases in these parameters[24].

Table 3: Parameters obtained from the Tafel polarization curves for the steel exposed to the bacteria *Erwinia genus*

Hours	E_{corr}/V vs Ag/AgCl	I_{corr}/µA cm^2	V_{corr}/µmy
360	-0.26	0.05513	0.044
408	-0.39	3.54	2.84
528	-0.67	7.36	5.87

In Fig. 5, the Nyquist plots are given where the resistive capacitive behaviour after interaction system are obtained with cultures of bacteria. In Table 4, the parameters found are shown for each of the evaluation times, for 360 hours, where a stainless steel obtained has high values Rp (about 94.7 kohmscm^2 in acid medium). This resistance value is attributable to the reaction kinetics of this material is exposed to an acidic environment. The items found by the equivalent circuit indicates that the corrosion phenomenon is governed by charge transfer, then highlighting the work of Cr in solid solution material and the important role of Si in the process of oxidation of Fe system alloys Cr-Ni, due to the formation of SiO_2 in the metal/oxide interface. The chromite formation is promoted by the continuous or discontinuous addition SiO_2 layer acting as a diffusion barrier[25].

The AISI 316L steel subjected for 480 hours shows a further decrease of 50% of the polarization resistance (about 39.5 kohmscm^2) in addition such values mean velocity values Rp relatively low corrosion, but increase considerably in comparison time of 360 hours. Likewise, the same spectrum behaviour in the two media study observed since the equivalent circuit has the same elements in both cases, highlighting the formation of a corrosion layer on the surface of the material it implies an increase in local capacitance said layer and thus increasing the resistance of the system[26].

Table 4: Parameters related to electrochemical reaction of the electrolyte and the metal substrate at different exposure times

360 Hours		
Parameter	**Value**	**Units**
R_{soln}	58.77	Ohms cm^2
R_{cor}	94.5e3	Ohmscm^2
R_{po}	47.92	Ohmscm^2
C_{cor}	25.30e-6	S*s^a
n	901.3e-3	
Cc	12.35e-12	S*s^a
m	650.5e-3	
480 Hours		
R_{soln}	50.82	Ohmscm^2
R_{cor}	39.50e3	Ohmscm^2
R_{po}	18.19	Ohmscm^2
C_{cor}	7.533e-6	S*s^a
n	961.8e-3	
Cc	97.15e-6	S*s^a
m	567.6e-3	
528 Hours		
R_{soln}	50.82	Ohmscm^2
R_{cor}	14.93e3	Ohmscm^2
R_{po}	177.01	Ohmscm^2
C_{cor}	398.7e-6	S*s^a
n	311.2e-9	
Cc	1.229e-3	S*s^a
m	683.4e-3	

For the same steel but subjected to 528 hours, it shows the same characteristic behaviour of steel evaluated 480 hours when comparing the Nyquist diagrams, observed according to the impedance spectrum and the equivalent circuit steels have the same evolution of corrosive phenomenon, which implies that the removal of the protective layer is generated after 360 hours therefore, after this period represents no major changes in the corrosive mechanism material[27].

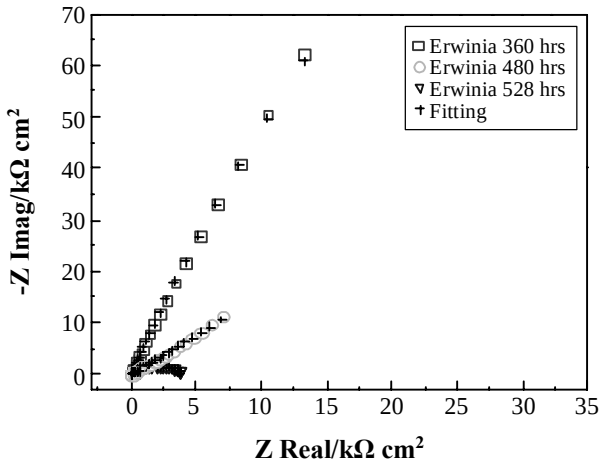

Fig. 5: Diagram Nyquist for the study of the reaction of the bacterium *Erwinia genus* evaluated versus time

The equivalent circuit is shown in Fig. 6. Here simulations was performed using nonlinear adjustment using software Gamry to adjust the data. The main parameter obtained by impedance spectroscopy has been the charge transfer resistance of the studied samples. From the parameters calculated by simulation, the transfer resistances for all samples were calculated. All samples have high transfer resistances, indicative of a very low intensity of corrosion. It is noted that the transfer resistance *Erwinia* samples generates a high value compared with the substrate at 480 and 528 hours[16].

The acid-producing bacteria produces biofilm as a defence mechanism to external agents, which is generated by the accumulation of organic and inorganic substances that act within the processes of energy metabolism as a carbon source for colonization of new bacterial genera. The generation of organic acids such as acetic, formic and lactic common in the metabolism process accelerates the localized corrosion due to affecting the kinetics of the cathodic reactions and/or anodic and chemistry of the protective layer due to the production of pyruvate and H_2 as an electron acceptor.

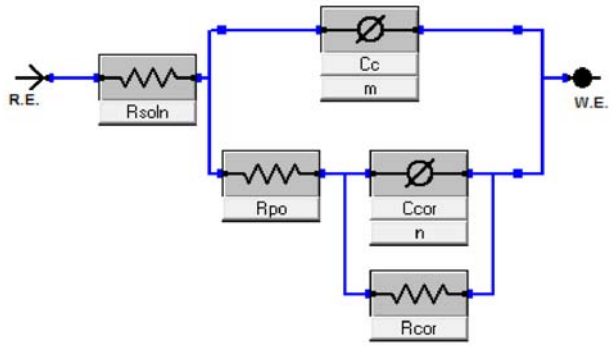

Fig. 6: The equivalent circuit corresponding to the evaluation of steel and presence of *Erwinia Genus* in function of time

In the graph of bacterial growth, it is observed that bacterial growth in the steel AISI 316 L, and the appearance of the exopolysaccharide matrix makes levels of the growth bacterial increases as acid production generates a niche for bacterial respiration and reproduction processes. For electrochemical analysis, it can infer that an increase in the corrosion current is generated, and in the corrosion rate which is influenced by bacterial growth.

CONCLUSION

The biofilm is generated by bacterial growth of *Erwinia*, which is slow and homogeneous on the surface of stainless steel AISI 316 L.

The acids produced by the metabolic processes such as lactic acid, formic react with carbon levels generating a biochemical process that generates favourable conditions for the growth of acid-producing bacteria.

By increasing the time of evaluation of steel AISI 316 L to the strain of the genus *Erwinia* is generated a corrosion potential lower, with respect to current and corrosion rate, which commits the chromium oxide layer.

The Nyquist plot demonstrates the reaction of alloying elements to the acids generated by the bacterial strain, corrosion it generated by transfer; however Cr and Si interface at the metal/oxide interface which acts as a diffusion barrier.

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