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Effects of Thiobacillus Ferrooxidans on corrosion of AISI 4140 Steel in presence of oil biodiesel

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In this paper we study the behavior of microbiologically induced corrosion by means of specie *Thiobacillus ferrooxidans* on AISI 4140 Alloy Steel with chromium and molybdenum high hardenability and good fatigue resistance, abrasion and impact influenced by *Thiobacillus ferrooxidans*. To determine the effect of process variables, speed and type of corrosion adhesion. The corrosion products formed on the surface of the samples using scanning electron microscopy were analyzed. The results showed the appearance of pinholes in the surface steel AISI 4140- after 24, 72, 120 and 168 hours of exposure in solution with the presence of *Thiobacillus ferrooxidans*, which was induced by the metabolism of bacteria, showing the presence of bacteria to accelerated the process of localized corrosion in the steel sample AISI 4140.

Keywords: *Thiobacillus ferrooxidans*, steel, corrosion, biodiesel.

1. INTRODUCTION

Among the advances to mitigate environmental impacts from fossil fuels management, the biofilms have emerged as an alternative in environmental issues and energy sustainability [1-3]. Excessive consumption of petroleum products has led to the rapid search for alternative fuels in recent years [4]. The biodiesel is chemically defined as a mixture of mono alkyl esters derived from long chain fatty acids and the mixture of diesel / biodiesel can be used as fuel [5-6]. However, the levels of wear and the corrosive effects of this compound on the metallic materials due to the reaction in the presence of water in the fuel microorganism which generates a biofilm wearing material [7-8].

The AISI 4140 alloy steel is an containing carbon, sulphur, silicon and manganese and that interact with water to generate a chemical or electrochemical reaction, which generates a specific microhabitat for growth of microbial populations which produce and release metal oxidation energy [9].

Within the wear processes are aerobic and anaerobic microorganisms, to expand change the chemical characteristics of the biofilm dynamics, which are modified and wear the material; among the factors that favour bacterial populations are: temperature, concentrations of carbon, nitrogen and the flow of oxygen concentrations and pH [6][10].

For this study the relationship between the microstructure and corrosion induced by different bacterial species, in a low carbon steel 4140, subjected to a biodiesel composition was evaluated.

2. EXPERIMENTAL

Specimens of medium carbon steel alloyed with chromium and molybdenum high hardenability and good fatigue resistance, abrasion and impact. The chemical composition of the base material, obtained by analysis of X-ray fluorescence, is presented in Table 1.

Table 1. Chemical Composition AISI 4140 steel.

Percentage	Compounds
0.38-0.43%	C
0.75-1%	Mn
0.80-1.1%	Cr
0.15-0.25%	Mo
0.15-0.35%	Si
0.04%	P
0.05%	S

Table 2. Culture medium composition.

Percentage	Compounds
20 g	Pancreatic digest of casein
5 g	Sodium chloride
10 g	dextrose
20 g	Agar
2 g	Sodium thioglycolate
1 g	Sodium formaldehyde sulphoxylate
2 mg	Methylene blue
1000 ml	Distilled water q.s.

For preparation of the specimens were polished with sandpaper different from the number 80 to 1200 until leaving with a mirror-like luster, each of the samples are subjected to the bacterial strain of *Thiobacillus ferrooxidans*¸ which are incubated at 35 °C, pH 6.5 and in a culture medium with the composition as evidenced in Table 2 [11].

To study the kinetics of growth of *Thiobacillus ferrooxidans* in the presence of biodiesel, monitoring for a period of 168 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].

The cell dry weight allowed to quantify the amount of biomass formed. They separated 20 ml of the growth medium, which were spun at 350 rpm for 20 minutes, the supernatant was discarded and the pellet was resuspended in distilled water the resuspended was placed in a previously treated pesafiltro, the resuspended was dried at 105°C until a constant weight. The weight was determined by the following equation.

To count the number of cells was performed for 168 hours every 24 hours 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 subidivided in a grid of 16 squares. Was used phase contrast microscope with a 40X objective for counting bacteria in only 5 of the 25 fields. It can be calculate the number of microorganisms in a sample, from the volume of the chamber and sample dilutions necessary. The equation (1) for this determination was [13]:

$$\frac{number\ of\ bacteria}{ml} = \frac{\sum \text{cells counted in the fields}}{4} * \frac{1}{dilution} * 10^{6} \qquad (1)$$

Electrochemical techniques were performed according to ASTM G1 and G3 regarding electrochemical tests [14-15].

All the electrochemical tests and microscopic were performed with a parameters of time 1, 3, 5 and 7 days incubation of Thiobacillus ferrooxidans in order to establish the bacterial growth in each sample and the wear caused to the steel surface [12]. Was used for the characterization of the surface a scanning electron microscope (SEM) Philips XL 30 FEG high resolution, equipped with EDAX EDX system whose resolution is 1 nm to 30 kV.

The electrochemical tests were performed in a Gamry model computer PCI 4, at room temperature, the immersion of the samples were made in a solution biodiesel. 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. 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 a 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. The working temperature of the trials was room temperature, with a controlled oscillation ± 0.1 ° C. To avoid contamination of the working environment, the specimens and the electrodes used were cleaned with acetone by ultrasound then with

98 % ethyl alcohol to remove bacterial debris and dried with hot air before being subjected to the Scanning Electron Microscope (SEM) and immersed in fluid.

3. RESULTS AND ANALYSIS

3.1 Growth kinetics

The species *Thiobacillus ferrooxidans* is morphologically gram- negative bacillus with polar flagellum. In the figure 1 shows the growth kinetics it observed having an incubation period of 168 hours in which the adaptation phase, exponential stationary and cellular death occur. The adaptation phase occurs during the first 24 hours in which cell counts are minimal reach a fraction of $1.2*10^6$, this due to the adhesion to the species needed in the steel [16].

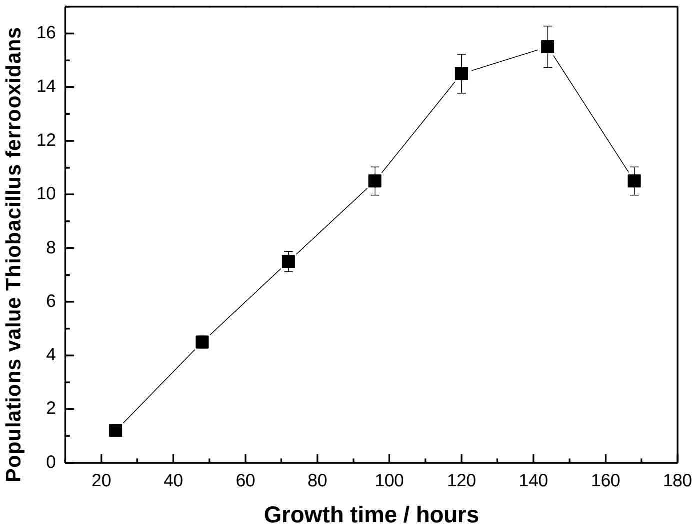


Figure 1. Kinetics bacterial growth on AISI 4140 steel, in function of time.

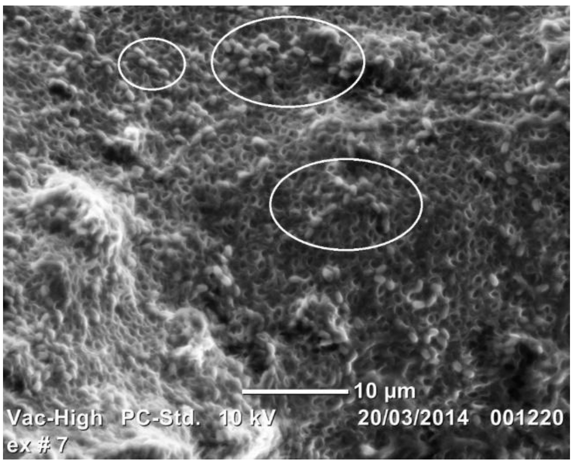


Figure 2. Micrograph of *Thiobacillus ferrooxidans* bacterial growth in AISI 4140 steel.

The period of cell replication in previous studies is given in a period from 300 to 350 hours in low carbon steels, This is because the bacteria of the genus are able to generate hydrogen sulfide via the sulfate reduction by dissimilation hydrogen sulfide. This acid combines with the iron salts to form an iron sulfide which is an important product of the corrosion[3,5] .

For this study the exponential phase is observed between 48 to 120 hours, the breeding period is accelerated by the reaction of biodiesel In the AISI 4140 steel surface of the cell doubling time accelerates biodiesel reaction by the metabolic processes of the bacteria and water the result is the ferrous ion reaction due to bacterial action is converted to ferric ion Fe3 + to the accept most of the electrons coming from the oxidation caused by the metabolic processes by the transfer of oxygen ions through a series of stages involving several mediator proteins located in the outer membrane that surrounds the bacteria; These electron transfer processes are coupled to the synthesis of ATP (adenosine triphosphate), which wears the surface of the steel [2,7].

The last stage occurs cell death due to dehydration and anoxia present in the culture medium, into account to the growth curve it evidenced a marked decrease and offspring in the curve.

Fig 2 shows the micrograph taken after 144 hours of incubation the present of colonies evidence that this covers the surface of the material, it observed streptobacilos chain, as which diplobacillus identifies the presence of iron for metabolic processes and cellular division.

3.2 Electrochemical impedance spectroscopy.

Fig 3 it observed the Nyquist diagrams corresponding to the steel is exposure to the taxa and saline versus time conducting evaluations at 24, 72, 120 and 168 hours, occurred phenomena that observed over time at the interface were indicated the increased protective effect at 24 hours of exposure early ages. The simulation results are included in the impedance spectrum using the circuit shown in Figure 4. As can be seen, there is a good agreement between experimental and simulated results. In Table 3 the values of the parameters used in the simulation are shown. The values of these parameters have been obtained using a complex nonlinear least squares program (CNLS) [17].

For all spectra is shown a definite semicircle at high frequencies and low frequencies is observed with a semicircle diameter less difficult to differentiate because the relaxation times are very similar. These semicircles indicate the interaction of two time constants. The semicircle found between 100 kHz and 100 Hz are related to the dissolution of the passive layer, reactions occurring at the surface due to the interconnected channels allowing the electrolyte to be absorbed on the surface of the steel. A low frequency of 1 Hz to 1 MHz, the behaviour is due to corrosion processes that are being generated at the interface between the ceramic layer generated by the surface oxide layer and the accessible superficial to the AISI 4140 steel, due to the changes observed in the diagrams of Nyquist because to a longer immersion are the value lower parameters of the electrochemical response indicating the capacity protection of this alloy analysed the high evaluation time is low, so that protection is required [18].

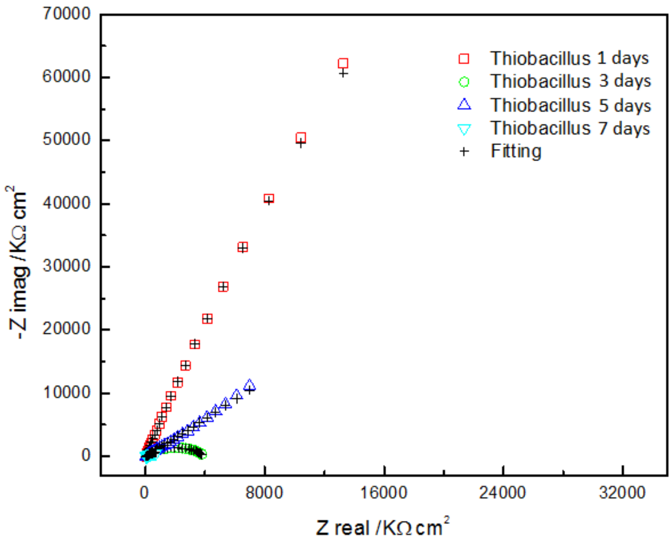


Figure 3. Diagram of Impedances (imaginary vs. real) of the AISI 4140 steel subjected to degradation by bacteria and evaluated at 1, 3, 5 and 7 days of immersion in biodiesel solution.

Table 3. Parameters used in the fitting of impedance data for substrate AISI 4140.

	R_Ω Ω cm^2	CPE_1 μF cm^{-2} $s^{-(1-\alpha1)}$	α_1	R_1 Ω cm^2	CPE_2μF cm^{-2} $s^{-(1-\alpha2)}$	α_2	R_2 10^3Ω cm^2
24 hours	58.21 (0.2%)	25.30 (4%)	0.90 (0.4%)	47.92 (5%)	12.35 (6%)	0.78 (0.3%)	941.5 (3%)
72 hours	50.82 (0.7%)	15.10 (0.9%)	0.73 (0.3%)	18.19 (6%)	97.15 (3%)	0.69 (0.5%)	79.50 (3%)
120 hours	62.61 (1.3%)	3.23 (1.4%)	0.77 (0.3%)	51.29 (4%)	144.15 (4%)	0.83 (0.6%)	3.94 (4%)
168 hours	58.59 (0.4%)	3.98 (2.4%)	0,82 (0.5%)	13.45 (2%)	398.7 (3%)	0.78 (0.3%)	3.12 (2%)

The equivalent circuit of Figure 4, corresponding to the Nyquist plots of Figure 3. In this paper a diagram contains two capacitor elements called "constant phase element of double phase" (CPE), which is independent of faradic reactions, which contribute with a pseudocapacitance (CPE1 + CPE2) to the total impedance of the system. Furthermore, in this electrochemical cell there is also an electric resistance, associated with the electrolyte resistance (Rs), which also will become clear in overall impedance of the system, this resistance corresponds to the value that contains the saline solution. The CPE1 coupling - R1, predominates in the higher frequencies, may be caused by the oxide layer while the CPE2 - R2 coupling, controls the low frequencies and this characterize the properties of the interface of the layer oxide/layer accessible.

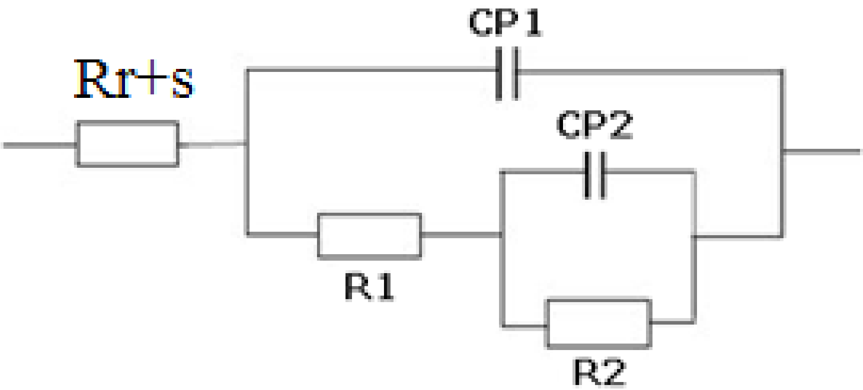


Figure 4. Equivalent Circuit generated used to fit the data for impedance of steel AISI 4140, under evaluation of the time function.

3.3 Polarization curves

In the Figure 5 is presents the results of potentiodynamic polarization corresponding to the AISI 4140 steel Tafel exposed to strain specie *Thiobacillus ferrooxidans*. One can observe the variation in corrosion potential (Ecorr) to the evaluation time.

It is generally observed that when the exposure time to the bacteria is short, the corrosion rate decreases to values nobler, this is because the bacterial adherence processes are minimal; also is necessary to time greater to 72 hours for the formation of the biofilm time. During the first 72 hours of biofilm formation creating sulphides generates a protection against corrosion caused by the salt solution. That is obtained after 72 hours of exposure potentials and achieve a more active Ecorr and these potentials are similar to 120 and 168 hours exposure and evaluation subsequent therefore it have is a greater tendency to corrode in saline as proceeds the evaluation in time function [19].

Referring to the corrosion current density (icorr) of AISI 4140 steel. It has the same behaviour found in Ecorr as a shorter evaluation the smallest corrosion current values are generated, These low values of the corrosion current density of AISI 4140 steel suggest that initially generates a stable oxide layer, then to advance the time of deterioration is observed that the bacteria produce a reduction in time to generate the passive layer.

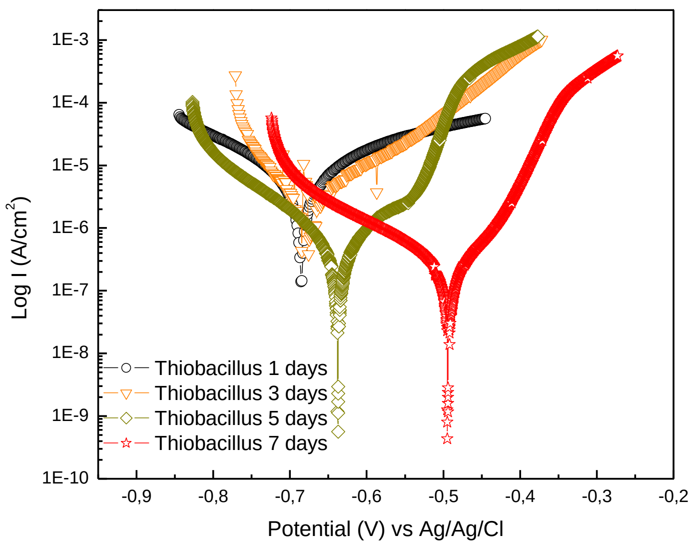


Figure 5. Tafel polarization curves of steel subjected to a solution of biodiesel with presence of bacteria.

Table 4. Electrochemical Tafel polarization parameters with the curves for the AISI 4140 steel, evaluated as a function of time.

	168 hours	120 hours	72 hours	24 hours
Beta A (V / Decade)	-137.58 e-3	353,2e-3	89,50e-3	113,6e-3
Beta C (V / Decade)	-64.97 e-3	537,9e-3	335,1e-3	161,3e-3
Icorr (µA)	5.553 µA	2,353 µA	1,706 µA	1,511 µA
Ecorr(mV vs Ag/AgCl)	-692.0	-674,4	-635,8	-472,9
Corrosion Rate (mpy)	25.35	10,58	7,672	6,795

In Table 4, the parameters are set to calculate the rate of corrosion, where the parameters found by means of polarization curves allow the use of the Stern's equation - Geary (Equation 2) to calculate the current densities of corrosion, where these differ depending on the current density, in addition is used to equation 3 for the calculation of B to from Tafel slope, the data obtained with the polarization curves Tafel can be seen in Table 4.

$$i_{corr} = \frac{\beta_a \beta_c}{2.303\, R_p\, (\beta_a + \beta_c)} \qquad (2)$$

$$R_p = \frac{B}{i_{corr}} \quad \text{Where} \quad B = \frac{\beta_a \times \beta_c}{2.3(\beta_a + \beta_c)} \qquad (3)$$

3.4 Microstructural analysis (SEM)

Fig 6 a micrograph of a AISI 4140 steel coupon before being subjected to bacterial attack is displayed. This micrograph was taken at 50 X and shows that has some scratches and surface points due to the polishing process of white metal.

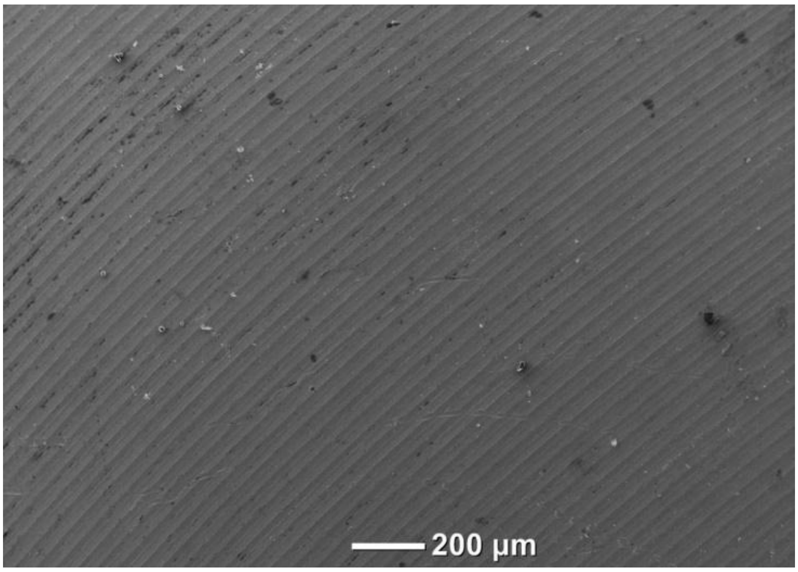
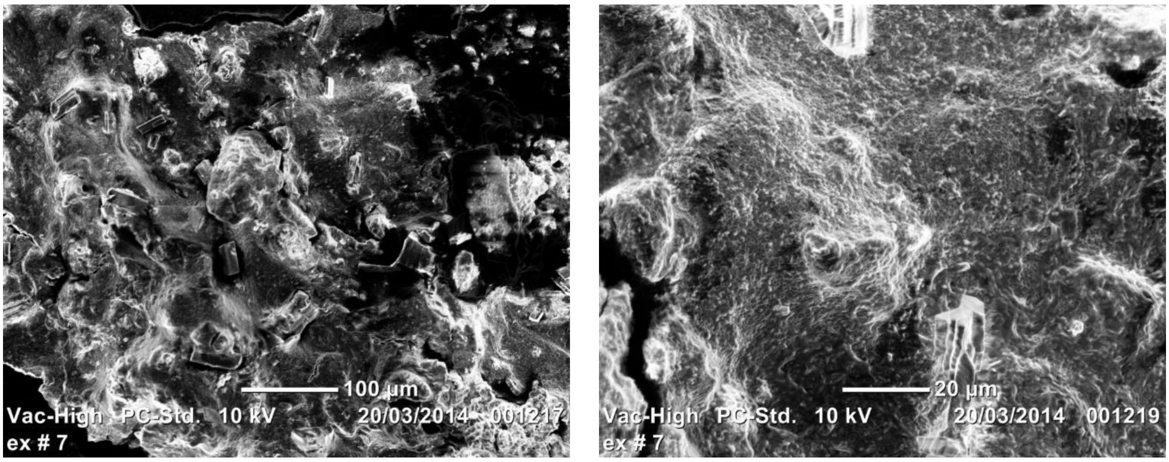


Figure 6. Microscopic observation of steel AISI 4140 in pre-incubation of the species *Thiobacillus ferrooxidans*.

In Figure 7a, the area of incubation of the species *Thiobacillus. ferrooxidans* on the surface of a AISI 4140 steel coupon it is manifested the corrosion products that is spread in a homogenous area located in the centre of the sample, in this micrograph certain clear areas where the bacterial inoculum is concentrated is observed, therefore a space the union of all its contour which diverges in a dark area to the next layer which are due to other corrosion products formed as progresses.

In Figure 7b, the surface is observed to observe more closely the sample surface to a 24 hours one day exposure of the specie *Thiobacillus ferrooxidans* the base metal microstructure consists of cavities in a homogeneous area with varying sizes seen at 1 micron (Figure 7b) showing a mechanism of uniform corrosion on the area of the surface of metal expressing loss of material by presenting corrosion.



(a) (b)

Figure 7. Micrograph showing a). film having concentration in the grain boundaries of the metal surface intergranularly b) mechanism of uniform corrosion.

4. CONCLUSIONS

The species *Thiobacillus ferrooxidans* wears the structure of the material AISI 4140 steel, this can be evidenced in accordance with the results presented by the electrochemical tests, this due to metabolic compounds having this bacterial genus, it follows according to the results a proportional relationship between the wear and exposure time occurs. The micrographs demonstrate bacterial adhesion and corrosion mechanisms loss material presented a uniform corrosion.

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