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Effect of Bacteria Medium and Biofilm Formation on the Electrochemical Behaviour of a Polished Austenitic Stainless Steel

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Résumé : Ce travail ambitionne d'étudier l'effet du milieu biotique et la microstructure de l'acier inoxydable AISI316L, et le comportement électrochimique et la formation des microcolonies de bactéries. L'étude électrochimique par des essais potentiostatiques, compare deux systèmes bactériens et une solution chimique aqueuse de FeCl₃. Les résultats indiquent une diminution de la vitesse de corrosion du spécimen d'AISI 316L quand le milieu de l'interface échantillon/bacteria est couvert par un biofilm. Les solutions bactériennes d'acidification (*Pseudomonas aeruginosa*, *Streptococcus thermophilus*) semblent être plus agressives que la solution de FeCl₃ de même acidité (pH 5.6). La modification du milieu électrochimique et les propriétés à l'interface sont des facteurs déterminants dans la résistance à la biocorrosion de l'acier inoxydable austénitique AISI 316L. La présence d'une couche de biofilm sur l'échantillon AISI316L a un effet retardateur de la cinétique électrochimique de corrosion. La résistance à la piqûration en présence de la couche de biofilm a été évaluée en enregistrant les courbes potentiostatiques jusqu' à 15 heures d'immersion.

Mots-clés – Acier Inoxydable, Activité Bactérienne, Essais Potentiostatiques, Microstructure, Biocorrosion.

Abstract: This work aims to study the effect of biotic medium and the AISI316L stainless steel microstructure, and the electrochemical behaviour and the formation of bacteria microcolonies. The electrochemical study via potentiostatic tests, compared between two bacterial systems and a chemical aqueous solution of FeCl₃. The results indicate a decrease of corrosion rate of the AISI 316L specimen when the interface specimen/bacteria medium is covered by a biofilm. The acidifying bacterial solutions (*Pseudomonas aeruginosa*, *Streptococcus thermophilus*) seems to be more aggressive than FeCl₃ solution with same acidity (pH 5.6). The modification of electrochemical medium and the interface properties seems to be an interesting way to improve the biocorrosion of the austenitic stainless steel. The presence of a Ps.a biofilm layer on AISI316L specimen has an effect on relaying the electrochemical kinetic of corrosion. The resistance to pitting in the presence of the biofilm layer was evaluated by recording the potentiostatic curves upto 15 hours of immersion.

Keywords – Stainless Steel, Bacterial Activity, Potentiostatic Tests, Microstructure, Biocorrosion.

I. INTRODUCTION

Austenitic stainless steels are widely used as a biomaterial in implants, and also in food industry and water treatment pipes where biotic medium are involved. It is well known that the electrochemical proprieties of austenitic stainless steels depend on their microstructure and the chemical composition, rich in chromium and nickel. In biotic medium, microorganism activity can provide the development of a biofilm on the surface of the steel and subsequently modify the electrochemical behaviour [1].

AISI 316L is the most popular grade of austenitic stainless steel. It is used as a structural materials in implants which are sensitive to pitting and intergranular corrosion at the metal surface, since their good corrosion resistance, good ductility, and excellent weldability [2]. During exposure to biotic environments, the protective surface oxide inherent to 316L is not stable. Opportunistic pathogens bacteria such can infect and subsist in the environment in which stainless steel structure is inserted [3]. Their life includes stages where the cells are associated and form a biofilm on the metallic surface. The formation of such interface communities and their content of some chemical agents like oxygen and H₂O₂ [4, 5] are the cause of many persistent damages on the metallic structure. Microscopic analysis [6, 7] has indicated that biofilm formation occurs in a sequential process of (i) transport of microbes to a surface; (ii) initial attachment; (iii) formation of microcolonies; and (iv) biofilm maturation [8]. The purpose of this paper is to study the electrochemical behaviour of AISI 316L stainless steel in bacterial mediums and also establish a relation between bacteria activity and pitting corrosion phenomena.

II. MATERIALS AND METHODS

The material used in this investigation is the AISI 316L stainless steel, according to the American standard nomenclature (Z3CND17-12-02). The chemical composition in weight percent is given in Table 1. The material was supplied by *Goodfellow* (France) in the form of plates of 1 mm in thickness. The elaboration by hot rolling and dynamic recrystallization involve a bulk polygonal austenitic microstructure with an average grain size of about 9,5 µm. Specimens for microstructure study and electrochemical tests were cut following the rolling direction, using a slow-speed diamond saw with liberal application

of lubricant for cooling. The areal dimensions of metallographic specimens are of 10X10 mm².

Table 1 : Chemical composition of the studied AISI 316L austenitic stainless steel.

%C	%Fe	%Cr	%Ni	%Mo	%Mn
0,03 8	64,46	17,5 6	11,8 9	2,48	1,49
%Cu	%Co	%S	%Nb	%W	%V
0,24	0,15	0,01 7	0,01 1	0,05 2	0,04 1

For the microstructure study and the observation of the surface state before and after tests, a Euromax optical microscope supplied by a CCD camera and a JEOL JSM-6360LV scanning electronic microscope in SEM mode were used for SEM and EBSD/SEM micrography. Each metallographic specimen was cold-mounted into a quick drying resin and mechanically polished with successively finer grades of SiC emery papers then in order to obtain the mirror state.

To reveal the microstructure, we used a chemical etching in HCl (30mL), HNO₃ (40mL) and distilled water (40mL). Also, an electrolytic etching was needed to reveal the grain size; it contains HNO₃ (50mL) and distilled water (50mL) under 2volts for the duration of 30 secondes. Using image analysis and processing by Aphelion software, we calculate the grain size and the average grain size of our microstructure.

The electrochemical behavior of 316L stainless steel is carried out by means of polarization tests using a voltameter VoltaLab40, operational with a PGZ301 regulator and VoltaMaster4 software for data processing. All the tests were accomplished in aerated conditions. All solutions are at 37°C and an initial pH of 5,6. A circular and vertical working electrode (1 cm²) was placed at the middle of the cell under a platine electrode. The reference electrode was a KCl-saturated calomel electrode (Hg/Hg₂Cl₂, E = +0,245 V_{/SHE}) set with a Haber-Luggin capillary. All working electrode potentials are given versus this reference electrode.

The corrosion tests are accomplished into two kind of acidifying aerobic bacterial solutions: *Pseudomonas aeruginosa* noted *Ps.a.*, *Streptococcus thermophilus* noted *St.th* and a FeCl₃ 10⁻⁸ mol/L solution. Composition of biotic mediums is given in table 2. The incubation of 15 hours in an organic nutritive solution is required to arise the bacterial activity on the surface of our

material and decrease the pH next to 5,6. The resulting corrosion behavior was quantified by means of polarization logarithmic curves. Specimens exposed to the biotic tests were polished with 1200 grade emery papers to obtain an average roughness of about 1µm, which aims to improve the adherence and development of microcells and biofilm.

The following experimental sequence was used: (i) Study of the metallographic state of the material ; (ii) Measurements of the corrosion potential (E_{corr}), corrosion current (I_{corr}) and the polarization resistance (R_p) ; and (iii) Recording of the potentiostatic curves $E - \log |i|$, from -1000 to 2000 mV versus E_{corr} with a sweep rate of 1mV/s.

Table 2 : Composition of the electrolytic solution used in the study.

Medium	Composition
Bacterial solution	Ps.a with biofilm in organic solution based on glucose (5gr/L)
	Ps.a without biofilm in organic solution based on glucose (5gr/L)
	St.th without biofilm + biofilm in organic solution based on lactose (product of fermentation)
Chemical solution	FeCl ₃ aqueous solution (10 ⁻⁸ mol/L)

III. RESULTS

Since grain boundaries are the first region of the metallic surface which reacts in answer with its environment, we consider in this section results of microstructural and grain boundaries study. In figure 1 and 2, we distinguish the regular microstructure of our 316L stainless steel with the presence of many twins and some inclusions. Figure 3 is obtained using an electron back scattered detector and show the morphology and the crystallographic texture.

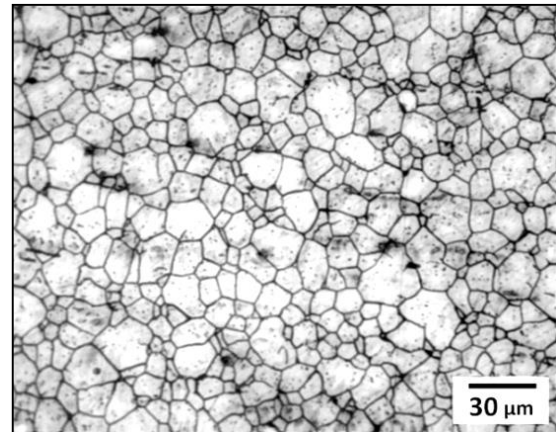


Fig. 1. Optical micrographs of 316L austenitic stainless steel using electrolytic etching.

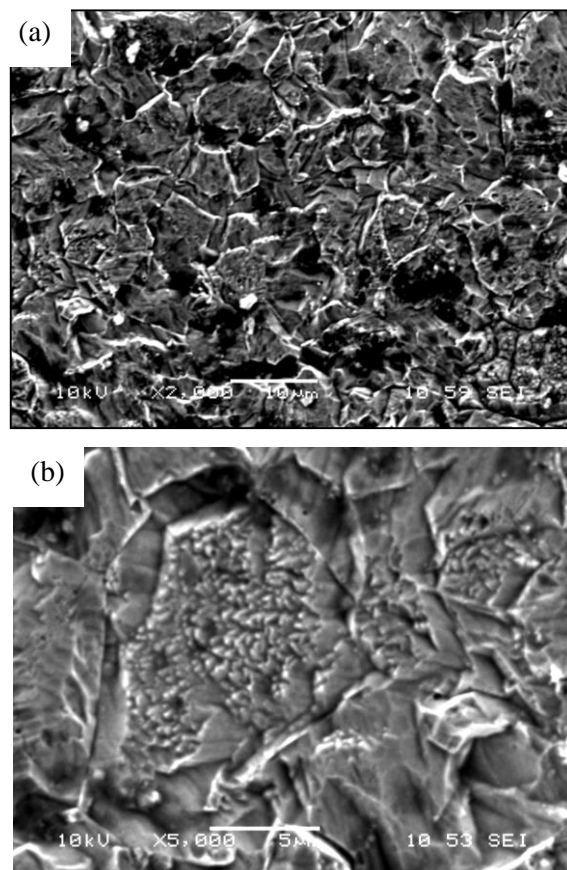


Fig. 2. SEM micrographs of 316L austenitic stainless steel specimen after chemical etching.

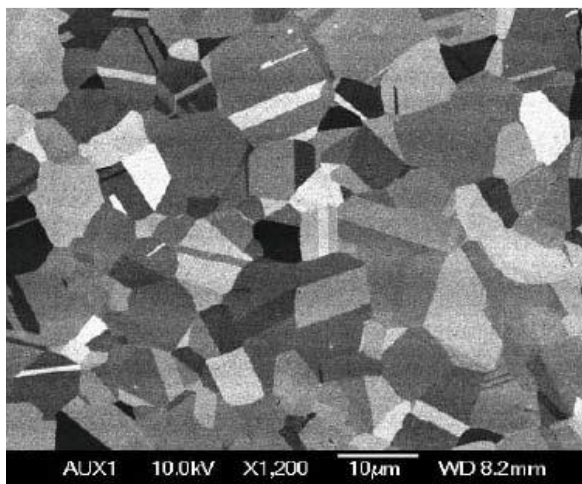


Fig. 3. EBSD/SEM micrograph of 316L austenitic stainless steel specimen.

The histogram in figure 3 is obtained by a statistical counting of grain size, computed on five different analysed images (Fig.1). It confirms the normal distribution of the grain size. In addition, the automatic micrography analysis lead us to estimate morphological parameters of grains such as “Circularity”, “Elongation”, and “log(length/width)” (figure 5).

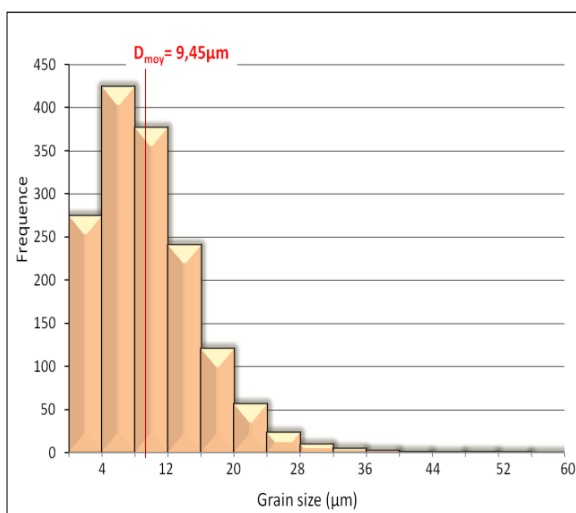
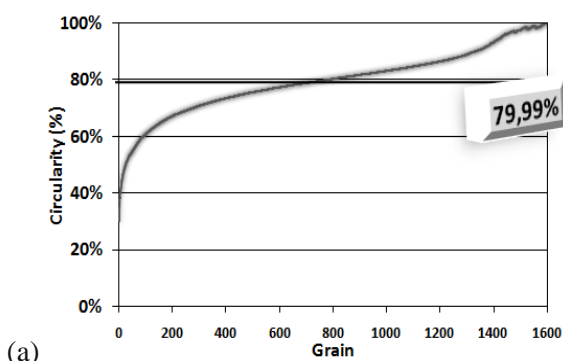
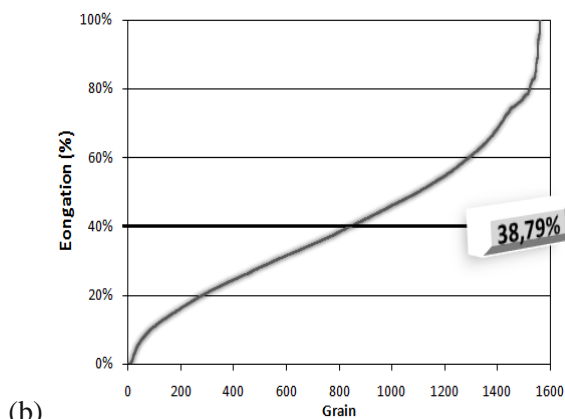


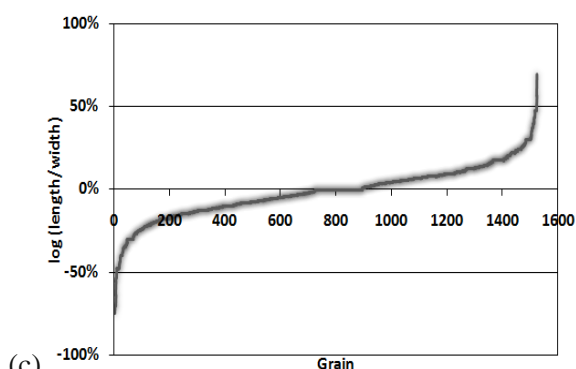
Fig. 4. Grain size distribution histogram of 316L specimen.



(a)



(b)



(c)

Fig. 5. Morphological parameters profiles of 316L grains; (a) Circularity, (b) Elongation, and (c) log(logth/width).

Electrochemical measurements lead to find out the parameters illustrated in table 3. The microscopic analysis has indicated that the biofilm formed by *Pseudomonas aeruginosa* under aerated conditions was found to be heterogeneous with mushroom-shaped microcolonies in which glucose was used as the carbon source. Bacteria seems to grow under static conditions in the wells of microtitre plates and may form a biofilm layer on the specimen.

Table 3 : Electrochemical parameters of polished 316L stainless steel in both bacterial and FeCl₃ chemical solutions.

Test condition	E _{corr} (V /SCE)	I _{corr} (A/cm ²)	Rp (Ω·cm ²)
Ps.a solution with biofilm	0,5783	4,58.10 ⁻⁵	2,70.10 ³
Ps.a solution without biofilm	0,5179	5,51.10 ⁻⁵	1,55.10 ³

St.th solution without biofilm	—	0,4765	$1,75 \cdot 10^{-5}$	$5,91 \cdot 10^3$
FeCl ₃ solution (10 ⁻⁸ mol/L)	—	0,4288	$5,73 \cdot 10^{-6}$	$1,50 \cdot 10^4$

After 15 hours of immersion in Ps.a medium (figure 6), we consider the presence of a thick biocorrosion layer of microcolonies of bacteria composed of microorganisms and metabolisms products reactions. This biofilm can form during the test after an immersion of 2hours. Pits and crevices are considerate after each test, surrounded by aggregate of microcells and organic substances (figure 7). No bacterial aggregates exist on specimen tested in St.th solution (figure 8) ; this kind of bacteria do not adhere on metallic surface but create great pits.

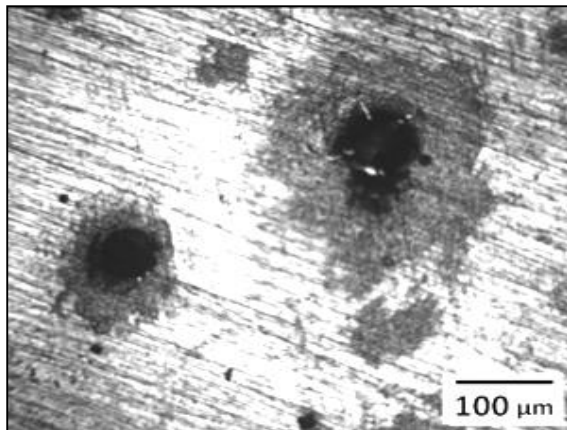


Fig. 6. Interface after potentiostatic test in Ps.a culture medium with an initial biofilm of formed during 15 hours on 316L sample.

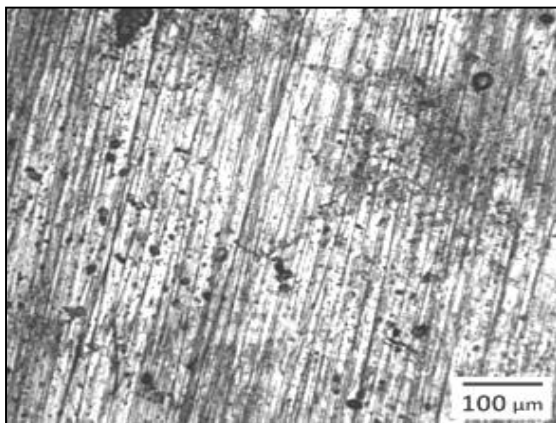


Fig. 7. Interface after potentiostatic test in Ps.a culture medium without an initial biofilm on 316L sample.

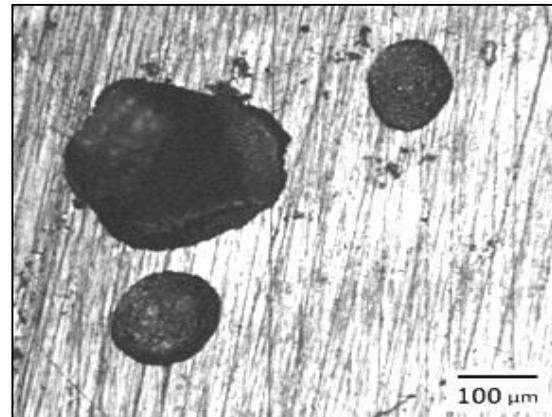


Fig. 8. Interface after potentiostatic test in St.th culture medium without biofilm on the 316L sample.

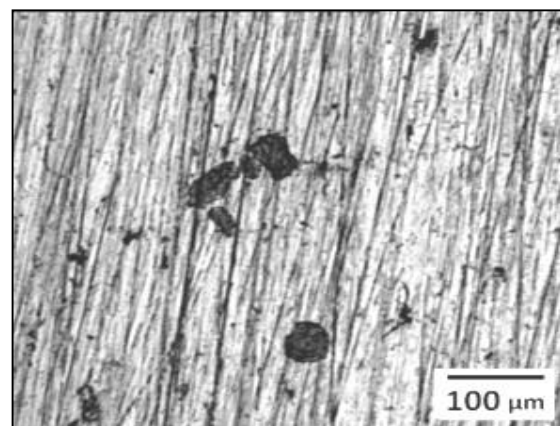


Fig. 9. Interface after potentiostatic test in FeCl₃ chemical solution of 316L.

Polarization in a chemical solution of FeCl₃ enhances pitting corrosion phenomena (figure 9). This result was found by other works [9, 10] is due to an anodic process located in the sensitive microstructural region, such as grain boundaries. After each essay, the measure of pH using a pH-meter shows an increase in the acidity of the solution as a consequence of the electrochemical process.

Table 4 : Variation of pH values after corrosion tests.

Solution	Initial pH	Final pH
Ps.a solution with biofilm	5,60	5,08
Ps.a solution without biofilm	5,60	5,28
St.th solution	5,60	5,71
FeCl ₃ 10 ⁻⁸ M	5,60	5,13

The superposition of polarization graphs in figure 10 and 11 illustrates an evolution in the electrochemical behavior according to the medium test. In fact, for the two samples tested in bacterial solution, the current measured both on the anodic and cathodic regions are of the same order of magnitude [11]. Consequently, electrochemical parameters measurements do not reveal significant differences between specimen without initial biofilm on it ($E_{\text{corr}} = -0,5783 \text{ V}_{\text{SCE}}$) and the one incubated 15 hours and covered with biofilm ($E_{\text{corr}} = -0,5179 \text{ V}_{\text{SCE}}$).

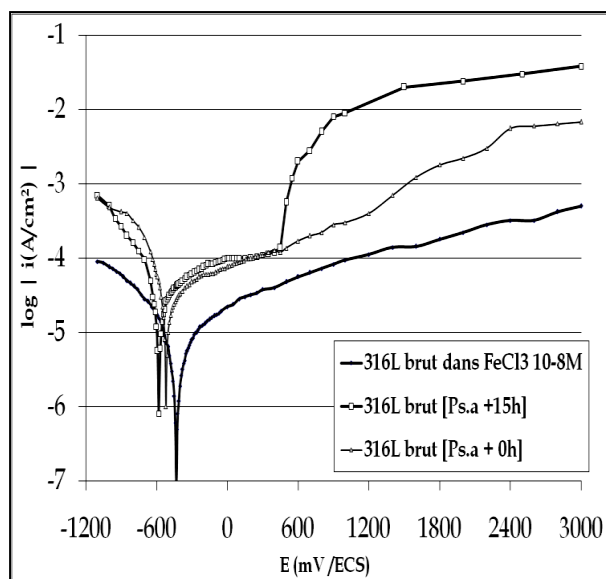


Fig. 10: Polarization curves in Ps.a medium compared with FeCl_3 solution.

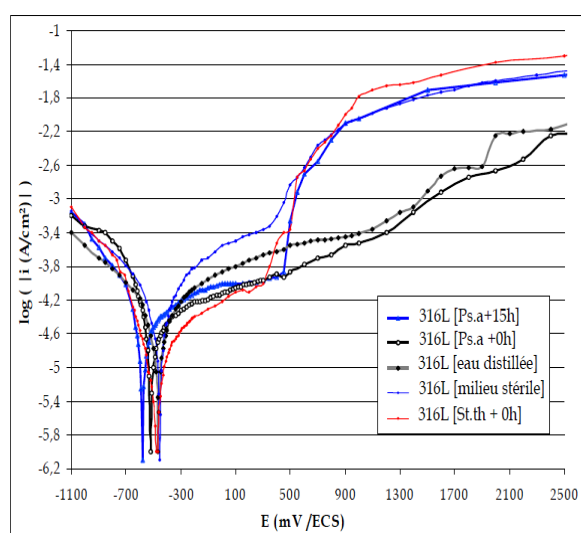


Fig. 11: Polarization curves in different medium.

IV. CONCLUSION

We have compared the action of chemical and fresh biotic media, on a stainless steel surface that is at the initial state.

Uniform corrosion occurs with FeCl_3 medium. The passive domain is large. The surface preparation by mechanical polishing causes an increase in the oxide layer on the studied specimen. The presence of a Ps.a biofilm layer on 316L specimen has an effect on the electrochemical kinetic of the corrosion phenomenon

The resistance to pitting in the presence or not of the biofilm layer was evaluated by recording the potentiodynamic curves upto 15 hours of immersion. The presence of a microcolonies of bacteria above the metal was noted using optical microscopy.

The biotic media, used without formed biofilm, seems to be more aggressive than the chemical media. Pitting which occurs on the surface is due to the biologic activity which can increase acidity at the interface between the steel surface and the medium. Electrochemical behavior can be affected by the composition and the volume of the corrosion products and the cyclic change of corrosion products volume as a function of time incubation.

REFERENCES

- [1] D. Haras, "Biofilms et altérations des matériaux : de l'analyse du phénomène aux stratégies de prévention, *Matériaux & Techniques* vol.93, (2005) 27–41.
- [2] J. Beddoes, K. Bucci, "The influence of surface condition on localized corrosion of 316L stainless steel orthopaedic implants", *Journal Of Materials Science : Materials In Medicine*, vol.10 (1999) 389-394.
- [3] E. Banin, M. L. Vasil, P. Greenberg, "Iron and Pseudomonas aeruginosa biofilm formation", *PNAS*, vol. 102 no. 31, (2005) 11076–11081.
- [4] D. Landolt, "Corrosion and Surface Chemistry of Metals", *EPFL Press*, 2007.
- [5] J. Buhagiar and H. Dong, "Corrosion properties of S-phase layers formed on medical grade austenitic stainless steel", *J Mater Sci: Mater Med*, vol.23 (2012) 271–281.
- [6] B. S. Stevenson, H. S. Drilling, A. P. Lawson, K. E. Duncan, A. V. Parisi and J. M. Suflita, "Microbial communities in bulk fluids and biofilms of an oil facility have similar composition but different structure, *Environmental Microbiology*", vol.13, no.4, (2011) 1078–1090.
- [7] L. Lemaître, N. Pénèbre, D. Festy, "Biodétérioration des Matériaux", *EDP Science*, 1998.

- [8] R. Schmidt, "Traité des Matériaux V.7 : Comportement des matériaux en milieux biologiques", *Presses Polytechniques et Universitaires Romandes*, 1999.
- [9] G. Béranger, A. Mazille, "Corrosion et Anticorrosion : pratiques industrielles", *Hermès Science Publications*, 2002
- [10] Y. Djemai-Zoghache, A. Isambert, N. Belhaneche-Bensemra, "Electrochemical behavior of the 316L steel type in a marine culture of microalgae (*Porphyridium purpureum*) under the 12/12 h photoperiod and effect of different working electrode exposure conditions on the biofilm–metal interface", *J Ind Microbiol Biotechnol*, vol.38, (2011) 1969–1978
- [11] J. Landoulsi, "Enzymatic Approach in Microbial-Influenced Corrosion: A Review Based on Stainless Steels in Natural Waters", *Environmental Science & Technologie*, no.42 (2008) 2233–2242.