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Contribution to the Study of Biotechnological Control Techniques Applied Against Biocorrosion Oil Installations

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Abstract*.The biological activity of oil extracted from green algae has long been known, but evaluation of bimolecular activities contained in this oil on an industrial scale, especially in the oil industry, was the objective of our research project. The first stapes of this study is to extract the essential oil from green algae marine Ulva Lactuca in our region, obtained by extraction/purification method based on hydro distillation and methanol extraction, the work performed at the laboratories of Sonatrach (SH/DTD/AUI/CEM). The second stapes is to evaluate the potential of these algae extracts on microbiologically influenced corrosion. After chemical-physic characterization of two bio naturals products A and B, we test their effectiveness as a bactericide on bacterial corrosion of carbon steel in water contained sulfate reducing bacteria by electrochemical analysis techniques (Open-circuit potential/OCP and Electrochemical Impedance Spectroscopy/EIS).*

Keywords*: biocorrosion, electrochemical, algae, FTIR, EIS*

1.Introduction

Plants offer a real potential for research of new molecules with inhibitory activity used for therapeutic purposes [1,2]. Few plant species are known and only a minority of them is chemically explored [1]. The marine environment is an infinite source of active molecules original chemical structure, such as green algae whose biological activity has long been known [3-5]. Many studies highlighted the biological activity of metabolites extracted from algae [6,7]. The evaluation activity of the essential oil extracted from green algae marine *Ulva Lactuca*, harvested from the Algerian coast, applied against micro-biologically influenced corrosion in the oil industry, was the subject of this study.

2. Materials and methods

2.1. Preparation of algae extract

The *Ulva Lactuca* green algae were harvested in the month of April 2015, on the coast of Dellys-Boumerdes at the Mediterranean Sea waters in Algeria where it grows in abundance in the spring.

Two extraction methods were used in this study, the hydro distillation one which gives as bio natural product A and methanol extraction which gives as bio natural product B.

Characterization techniques of extracts product

Organoleptic and chemical-physic properties[#]

All properties such; *p*H, density, viscosity, appearance, color and odor were determined and recorded in Table 1.

Quality control analysis was performed in the Control and Fluid Treatment Laboratory at DTD/SONATRACH - Boumerdes - Algeria.

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Table1. Organoleptic and chemical-physic characteristic of bio natural's products A and B extracted from green marine algae *Ulva Lactuca*

Characteristic / Product	Aspect	Odor	Color	T°C	pH	Density g/cm^3	Viscosity at 20°C cP	Solubility	
Product "A"	Liquid	algal	colorless	21	7.14	1.001	1.40	Water Alcohol hydrocarbon	Soluble Soluble Insoluble
Product "B"	Liquid	algal	Green algae	21	4.67	1.002	2.35	water Alcohol hydrocarbon	Soluble Soluble insoluble

2.2. Infrared spectrum analysis

Analysis by infrared spectrum****** allowed us to characterize our product using spectrometer Shimatzu FTIR 8900 by formulating a drop extract of green algae on a KBr pellet. The IR spectrum were determined in Figure 1.

******This analysis was conducted at Chemical Laboratory at Science Faculty at M'hamed Bougara University of Boumerdes - Algeria.

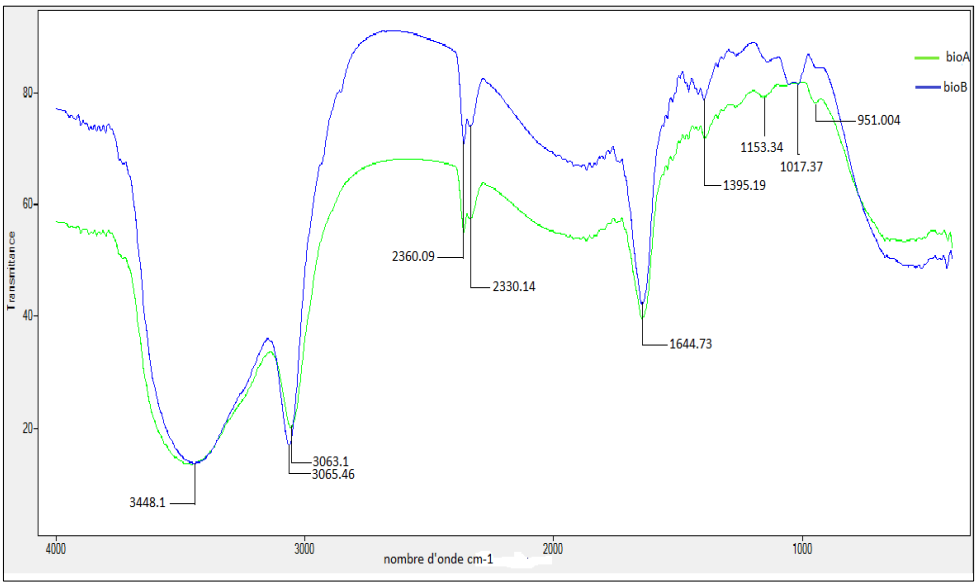

Figure 1. IR spectrum of extract green algae marine Ulva Lactuca by two extractions methods

In the literature, the use of green plants as inhibitors of metal corrosion is explained by its high molecule having O – H groups, C = O and C = C groups in their chemical structure. Hamdy and El-Gendy (2013) studied the inhibitory action of extract of Henna Lauzenia Inermis and its major constituents on the steel corrosion. The inhibitory efficacy of the extract from the Asteraceae was evaluated on steel by Garai et al, (2012). Deng and Li (2012) used the Phillyrin and Verbascoside, active compound extract from Jaminum Diflorum sheet steel for protection against corrosion. Xanthyletin the active compound of the extract of leaves of Citrus aurantifolia was used as an inhibitor of steel corrosion by Saratha and al, (2009) [8-11].

All these works cited and others performed in the same context [12-17] reported high values of the inhibitory efficiency as a function of alcohol, esther, aldehyde, amine, ketone and acid; identified in chemical structures of compound majority extracts.

Our case confirms by Infrared spectrum analysis given by the Figure 1 and which has the following:

The spectrum presents an intense and broad absorption band around 3400-2500 cm^{-1} indicates the presence of hydroxyl group OH.

The spectrum has an intense absorption band around 1820-1660 Cm^{-1} indicated the presence of carboxyl group C = O.

The spectrum presents a medium intensity absorption band around 3400-3300 Cm^{-1} indicated the presence of NH group, making the presence of amine.

The spectrum has an average intensity of absorption band around 1650-1450 Cm^{-1} indicated the presence of the double bond C = C.

The spectrum shows an absorption peak very fine average intensity at 2250 Cm^{-1} which confirmed the presence of nitrile compound and the benzene ring.

According functions present in this spectrum confirming that the bimolecular extracted from green algae marine that is responsible for protection of carbon steel against microbiologically influenced corrosion is an amine or amine derivative.

2.3. Selection of bacterial strain tested

The bacterial strain tested Sulfate reducing bacteria say SRB is isolated from industrial water injection wells in oil field Hassi Messaoud - BRN in southern Algeria. These bacteria were incubated at 37°C in a specific culture medium for proliferation prepared according to the API RP 38-1999 Standard [18].

2.4. Efficiency test of bio natural products extracted from *Ulva Lactuca* by bacteriogical test

Pre-culture was prepared with water injection which is enriched with chemical constituents of specific culture medium of SRB. The pre-culture is then distributed in bottles of penicillin, bubbled with nitrogen and sterilize at 120°C for 20 min at 1 bar pressure. After sterilization the flasks are left twice in series of four bottles and each series is inoculated with 1 mL (10%) SRB strain. Incubation of pre-culture is carried out at 37°C for 48 h.

Bio naturals products A and B extracted from Ulva Lactuca is then added to the vials prepared to selected concentrations of 0, 5, 10, 15, 20, 30, 50, 100, 150, 200, 250, 300, 350 and 400 ppm.

Then incubated at 37°C until 28 days. The procedure of reading penicillin bottles during incubation period. Upon visual inspection of bottles, two case:

1st case: bottle positive (+): medium turning from black by SRB, which shows that the bio natural product is ineffective.

2nd case: Bottle negative (-): not turning to black by SRB, which shows that the bio natural product is effective.

We note that contamination by SRB in entreated medium is 10^4 germs/mL, but after injection of bio natural's products extracted from green algae marine *Ulva Lactuca* A and B with differences doses, no contamination were recorded during 28 days of incubation at 37°C. The results are given in annex A.

Annex A. Results of monitoring treatment efficacy anti corrosion by bio natural products "A" and "B" in liquid medium

Injection Date	Tested Product	Incubation period (day) bacterial sowings made on the injection																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
		Concentration of SRB (germs / mL) after treatment with bio natural products A and B extracted from green Algae marine *Ulva Lactuca*																											
0 ppm 18.03.2015	SRB (Blank)	0	0	0	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4
5 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

20 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150 ppm 18.03.2015	Product "A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product "B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200 ppm 18.03.2015	Product"A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product"B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
250 ppm 18.03.2015	Product"A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product"B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
300 ppm 18.03.2015	Product"A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product"B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
350 ppm 18.03.2015	Product"A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product"B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400 ppm 18.03.2015	Product"A"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Product"B"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

3. Results and discussions

3.1. Electrochemical tests

The studied corrosion reaction occurs at the interface of electrode. The material used as the working electrode is carbon steel shade API 5L X60. Table 2 gives as the chemical composition of the steel sample.

Table 2. Chemical composition of carbon steel (in mass %)

Elements (%) / Sample	C	Si	Mn	Ni	Cr	P	S	Mo	Cu	Fe
API 5L X60	*0.22*	*0.23*	*1.31*	*0.044*	*0.05*	*0.013*	*0.01*	*0.016*	*0.06*	*98.047*

Evaluation of corrosion rate in terms of carbon steel time in the presence of SRB (test blank - without treatment)

Table 3 and Figure 2 represent corrosion rate evolution of carbon steel immersed in injection water enriched and contemned by 10^2 germs/mL of SRB versus time of incubation.

Table 3. Results of monitoring corrosion rate of carbon steel immersed in contaminated water injection by the SRB versus time of incubation at 37°C

Time, (h)	4	8	24	48	72	96	120	144	168	240	336
Corrosion Rate (mm/year)	0.050	0.130	1.837	3.366	7.301	8.209	5.345	4.069	2.959	1.506	0.106
Corrosion Rate (mpy) (Mil par year)	2.001	5.146	72.31	132.5	287.4	323.2	210.4	160.2	116.5	59.29	4.172

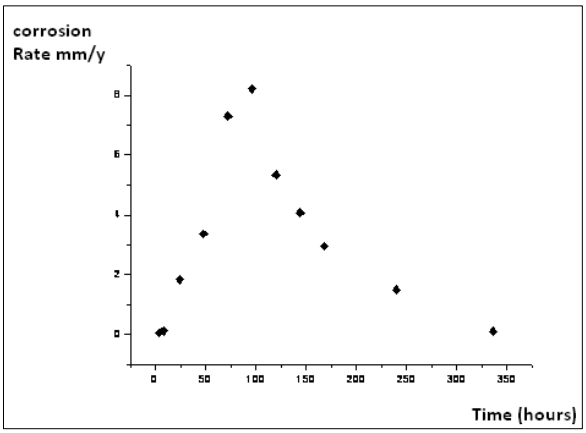

Figure 2. Monitoring evaluation of corrosion rate of carbon steel submerged in a contaminated water injection by the SRB versus time of incubated at 37°C

The shape of the curve increases as a function of incubation time of the SRB. A corrosion rate recorded during *24 to 48 h* is greater than *3.366 mm/year* or *132.5 mpy*, after 72 h the steel corrosion rate reached a maximal, *8.2 mm/year* or *323.2 mpy* was detected at the 4th Day or *96 h* of incubations. A decrease in the corrosion rate was recorded from the 5th day. We note that this curve is identical to the bacterial growth curve, the rate of carbon steel corrosion is maximal when the bacteria are in the exponential phase or their growth rate μ is maximal.

For low speeds recorded, the 7th and 8th day of incubation, are due to the low concentration of SRB in water due to the depletion of minerals and trace elements necessary for their growth and the accumulation of waste metabolisms that become toxic for bacteria and leads to cell autolysis.

Monitoring the open-circuit potential *(OCP)* is intended to achieve the stabilization time of metal-solution interface. Table 4 gives as the variation of potential for a shade carbon steel electrode API5L X60 in the presence of SRB in injection water before and after treatment with bio natural's products A and B.

Table 4. Corrosion Potential of steel measured in the various environments in the presence of SRB (10^2 germs/mL) in medium treated with bio natural's products A and B

Electrolysis	**Incubation time (h)**	**Stabilization Time *(s)***	**OCP *mV/ECS***
Injection water contemned by 10^2 germs/ml SRB	72	4500	- 774
Injection water contemned by SRB and treated with 5 ppm of bio natural Product A	120	4500	- 643
Injection water contemned by SRB and treated with 30 ppm of bio natural product A	120	80000	- 335
Injection of water contemned by SRB and treated with 5 ppm of bio natural product B	120	4500	- 696
Injection of water contemned by SRB and treated with 30 ppm of bio natural product B	120	80000	- 300

Potential measurement is followed for an immersion time of 72 h for the blank sample and 5 days for treated one with bio natural product. The corrosion potential recorded in the presence of SRB equal to (-774 mV/SCE); According to Pourbaix's diagram [12].

This potential is identical to that of steel immersed in a solution contaminated by SRB so that we localized corrosion and iron dissolution.

For values of (-696 to -300 mV/SCE) stored in Table 4 and obtained in the presence of treatment with bio natural products A and B; are noble and tend toward more positive potentials.

The film formed on steel area prevents from oxide reduction reactions and anion exchange to take place, so that SRB metabolism was blocked and bio natural's products adhere to the area of steel by forming a protective film.

Bacteria present in an aqueous medium rich of nutriment necessary for their metabolism and development behave according to the potential Pourbaix's diagram iron-*p*H [19]. This diagram describes the relationship between the corrosion influenced by microorganisms (MIC) and the electrochemical characteristics of the steel corrosion.

Carbon steel alloys present as micro-heterogeneous material which confers in a different electro-chemical behavior by corrosion metabolism of bacteria present in the medium and their effect on the material. Forming a biofilm layer on the area of working electrode followed by an attack of corrosive medium (rich in chlorides and sulfates water injection) caused corrosion by SRB which explains the recorded values of potential corrosion as a function of time (-774 mV/SCE); Forming a protective layer after injection of the biomolecular on our steel explains the recorded values of corrosion potential versus time ranging from (-696 to -335 mV/SCE).

Scanning electron microscopy (SEM) was used for characterizing the surface morphological properties of SRB biofilm was presented in Figure 3 and observations of protective film formed by bio natural's product A and B extracted from green algae marine Ulva Lactuca over steel surface, were presented in Figure 4 and Figure 5.

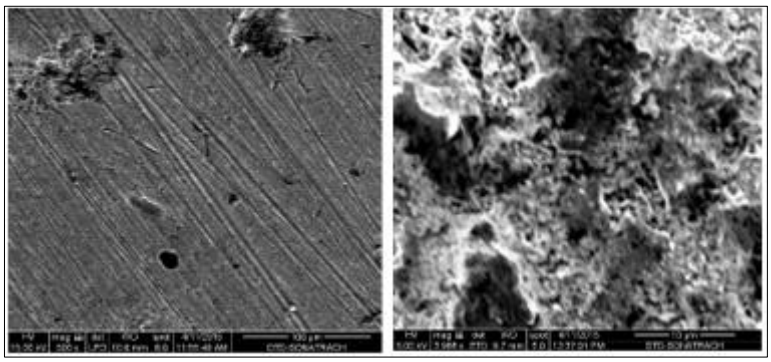

Figure 3. SEM micrographs of steel immersed in contemned medium by the SRB for 10 days

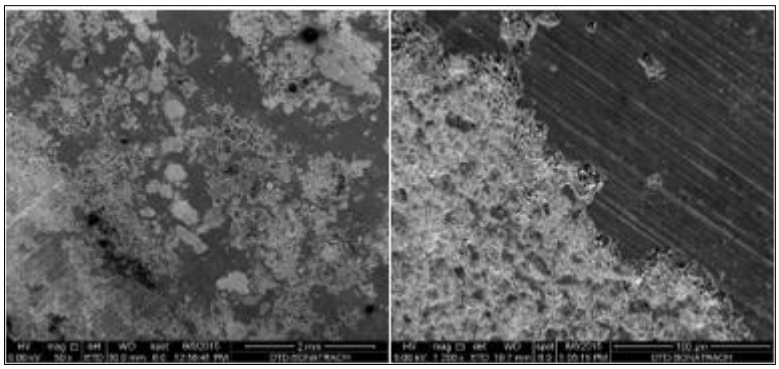

Figure 4. SEM micrographs of steel immersed in contemned medium by the SRB treated with bio natural's product A with 30 ppm

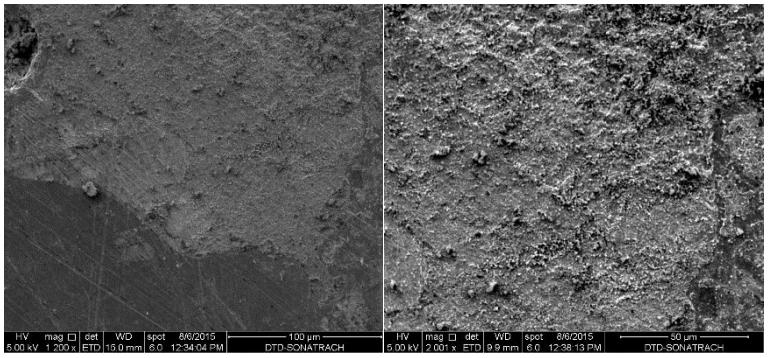

Figure 5. SEM micrographs of steel immersed in contemned medium by the SRB treated with bio natural's product B with 30 ppm

3.2. Electrochemical impedance spectroscopy (EIS)

Electrochemical impedance spectroscopy technique was used to confirm corrosion of carbon steel immersed in injection water contemned by SRB with presence and absence of Ultra Lactuca's extract product used as treatment against microbiologically influenced corrosion. This technique has the advantage to minimize disruption of metal-solution interface, as the superimposed alternative signal courant to continue signal courant is low (± 10 mV). The impedance curves are shown by the Nyquist plots and Bode.

The intersection of semi-circles on the Nyquist plots with the real axis (Z') represents the solution resistance (R_s) at high frequency and the charge transfer resistance (R_{tc}) at low frequency which give as polarization resistance (R_p) [20].

3.3. Test with bio natural product A

Table 5. Electrochemical parameters obtained by EIS of carbon steel immersed in a medium treated with the bio natural product A at various concentrations

Concentrations (ppm) / **Parameters**	0 (Blank)	5	10	15	20	30
OCP (mv/ECS)	-725	- 664	- 660	- 644	- 460	- 460
R_s *(K Ω.cm²)*	0.0088	0.0082	0.0179	0.0176	0.0980	0.0980
R_{tc} *(K Ω.cm²)*	0.177	3.837	21.167	35.549	295.862	433.722
R_p *(K Ω.cm²)*	0.186	3.846	21.185	35.567	295.96	433.82
C_{dl} (µF /cm²)	1900	6000	190	190	190	190
Maximum phase Angle Θ (deg)	-60°	-48°	-46°	-50°	-45°; -20°	-44°; -55°

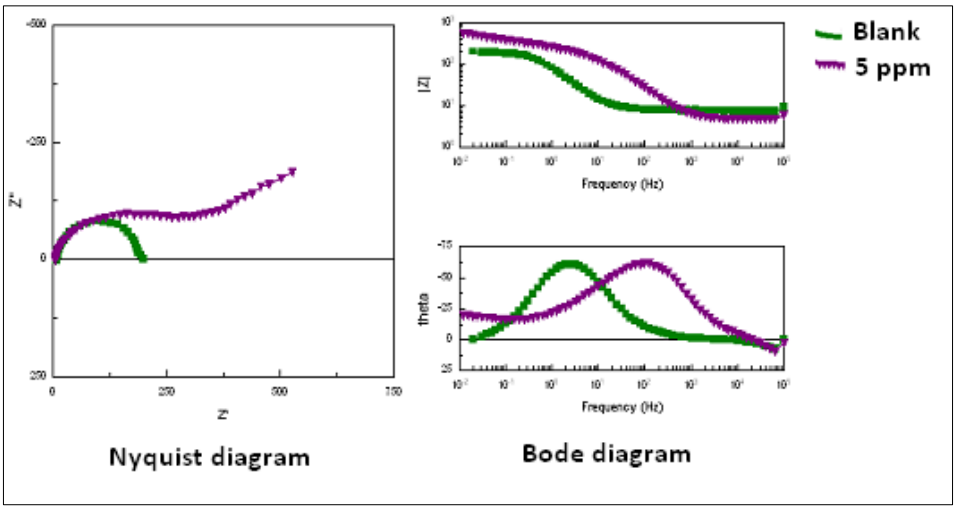

Figure 6. Nyquist and Bode diagrams obtained by EIS of steel immersed in contemned injection water by 10^2 germs/mL of SRB (Blank) and treated with 5 ppm of bio natural product A

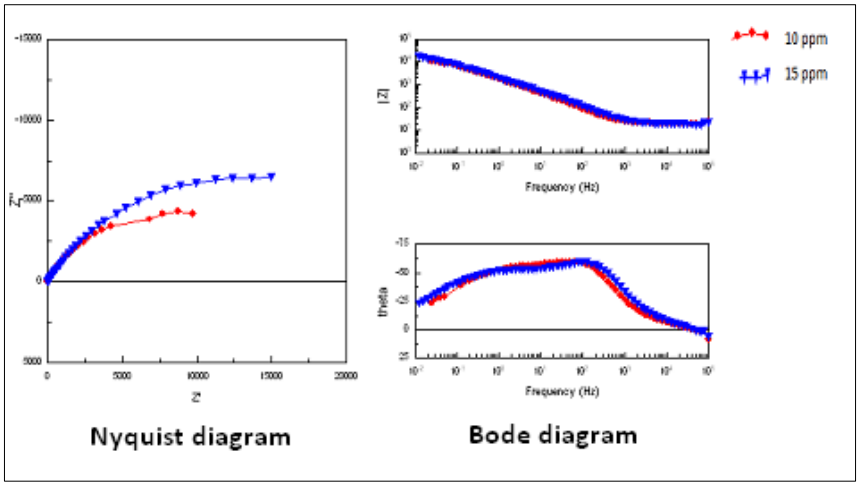

Figure 7. Nyquist and Bode diagrams obtained by EIS of steel immersed in contemned injection water by 10^2 germs/mL of SRB and treated with 10 and 15 ppm of bio natural product A

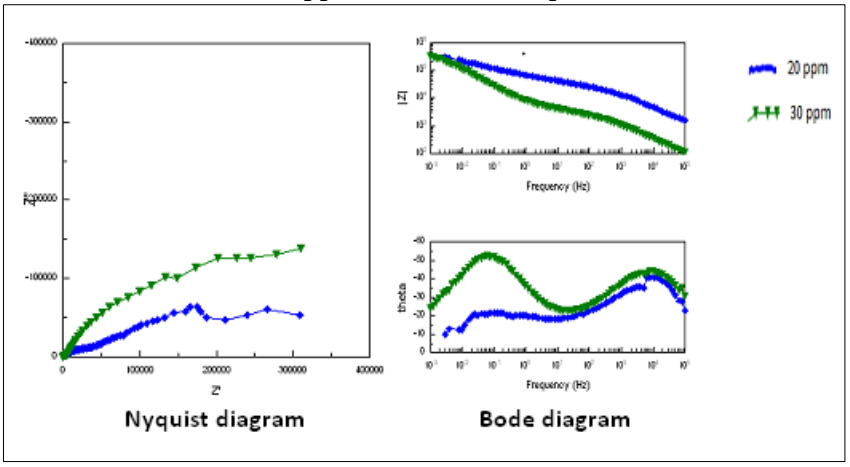

Figure 8. Nyquist and Bode diagrams obtained by EIS of steel immersed in contemned injection water by 10^2 germs/mL of SRB and treated with 20 and 30 ppm of bio natural product A

From Figure 6 to Figure 8 is found that the semi-circles of impedance diagram showing the control of corrosion steel by the charge transfer process. Thus, the resistance solution is low for contemned medium with SRB.

The impedance parameters given in Table 5 show an increase in charge transfer resistance, a decrease in the values of the capacity of double layer C_{dl} according to the increase in the concentration of bio natural product A; these results indicate that the algae extract obtained by the hydro distillation method, inhibits the process of microbiologically influenced corrosion of steel by adsorption mechanism. C_{dl} is inversely proportional with polarization resistor R_p, resistance of material increases when a protective film was formed on metal surface and the electron cloud ion exchange (anion, cation) taken place and the double layer has a fine appearance order values of 10^{-5} to 10^{-7} Farad/cm^2, confirming reaction of biomolecular by inhibiting adsorption mechanism to the metal surface.

3.4. Test with bio natural product B

Table 6. Electrochemical parameters obtained by EIS of carbon steel immersed in a medium treated with the bio natural product B at various concentrations

Concentrations ppm / Parameters	0 (blank)	5	10	15	20	30
OCP (mv/ECS)	-725	-697	-675	-660	-644	-485
R_s *(K Ω.cm^2)*	0.0088	0.0155	0.0179	0.0177	0.0177	0.207
R_{tc} *(K Ω.cm^2)*	0.177	2.448	11.678	29.700	40.04	399.01
R_p *(K Ω.cm^2)*	0.186	2.464	11.696	29.718	40.057	399.22
C_{dl} (µF /cm²)	1900	340	140	170	200	270
Maximum phase Angle Θ (deg)	-60ºC	-48º	-46º	-50º	-45º; -20 º	-44º; -55 º

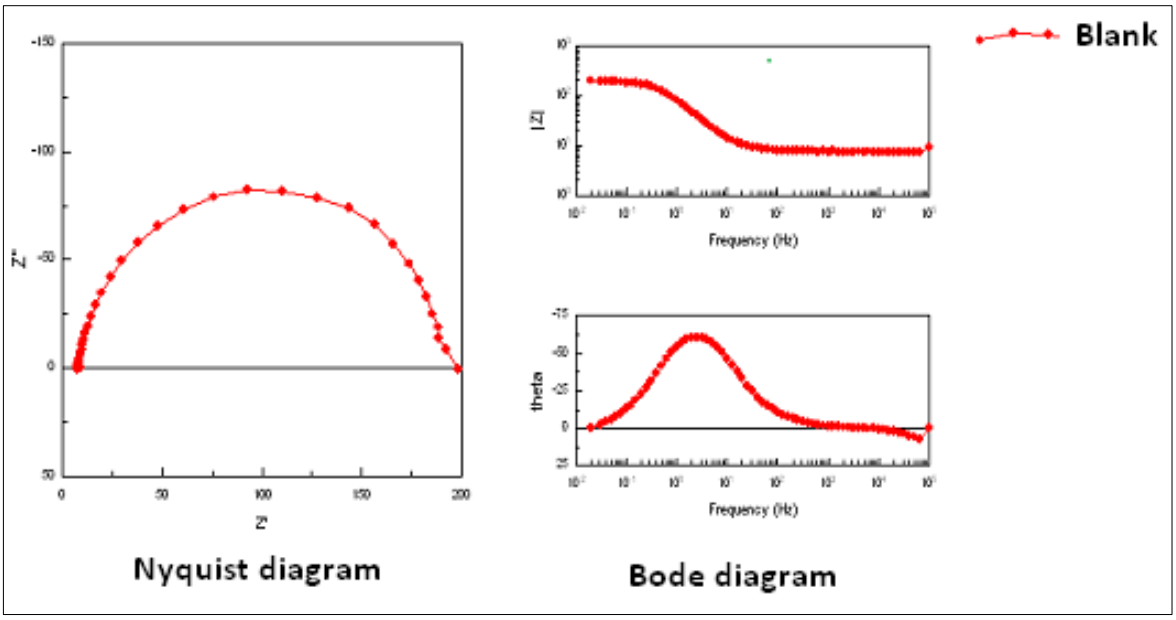

Figure 9. Nyquist and Bode diagrams obtained by EIS of steel immersed in contemned injection water by 10^2 germs/mL of SRB

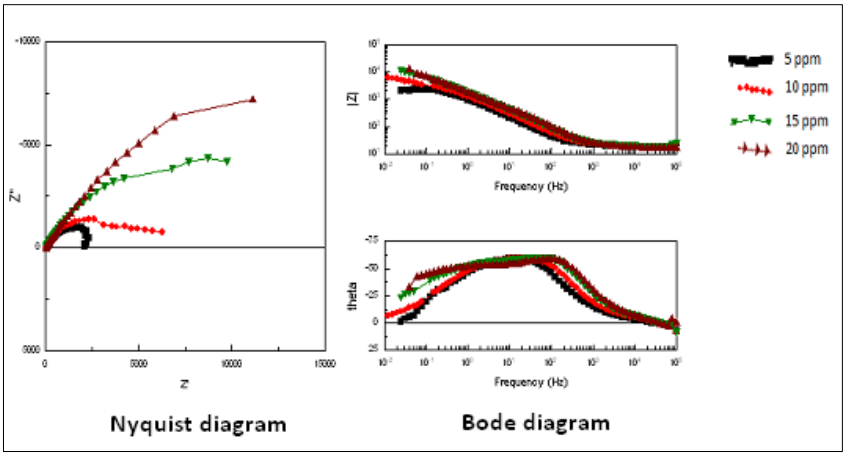

Figure 10. Nyquist and Bode diagrams obtained by EIS of steel immersed in a contemned injection water by 10^2 germs/mL of SRB and treated with various bio natural product B (5, 10, 15 and 20 ppm)

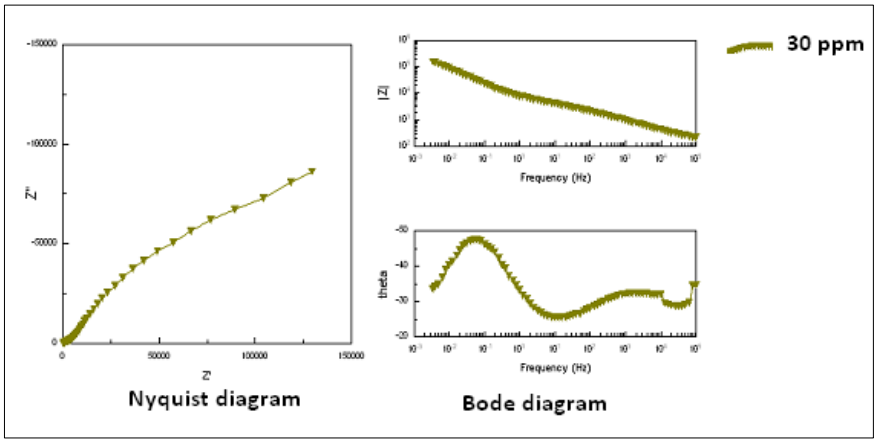

Figure 11. Nyquist and Bode diagrams obtained by EIS of steel immersed in contemned injection water by 10^2 germs/mL of SRB and treated with 30 ppm of bio natural product B

According to Figures 9, 10 and 11; we note that when bio natural product B was injected impedance diagram shows the same trend capacitive loops whose diameter increases with increasing concentration.

The results given in *table.6* indicate an increase in charge transfer resistance R_{tc} values and a decrease in capacity of double layer C_{dl} values according to the increase of bio natural product B concentration, this decrease is associated with the adsorption of biomoleculs of algae extract at steel surface.

Polarization resistance R_p of blank sample is *0.186 KΩ.cm²* after injection various concentration of bio natural product B, R_p values increase to *11.696 KΩ.cm²* with *10 ppm* and to *399.22 KΩ.cm²* with *30 ppm*. The capacities of the double layer are weak, values order are 10^{-5} Farads/cm².

The phase angle *θ* indicated was greater than -54° for each concentration, with respect to the dose of 30 ppm scanning steel surface was performed by two phase angles θ1 equal -32° and θ2 equal -49° confirming the presence of a protective Film formed on steel surface by bio natural product B extracted by methanol extraction method from green algae marine *Ulva Lactuca.*

4. Conclusions

The purpose of this study was the characterization of biofilm on carbon steel immersed in industrial injection water contemned by sulfate-reducing bacteria SRB. The main conclusions we reached are:

The injection water from the oil field Hassi Messaoud-BRN in south Algeria is rich by nutriments representing the various energy sources; such as carbon, sulfur, calcium, magnesium, these elements are necessary for enzyme activity and have a catalytic role of the bacterial cell.

Microbiological analysis of this water highlights sulfate-reducing bacteria with concentration of 10^4 germs/ml. The evolution of bacterial corrosion rate versus time incubation of steel immersed in injection water contemned by SRB has given us very high values in the presence of SRB from the 3rd day of incubation and decreases the 5th day.

The extraction of active moleculs of green algae marine *Ultra Lactuca* harvested from the Algerian coast; by two methods of extraction hydro distillation and methanol extraction yields two bio natural products A and B containing molecules responsible for the inhibition of bacterial corrosion by their protective potency of carbon steel against the effect of SRB.

An infrared spectrum analysis allowed us to identify the essential functions of biomoleculs acting as microbiologically influenced corrosion inhibitor, and acting either on the product microbiologically influenced corrosion in steel surface or on the SRB growth metabolism.

The use of electrochemical techniques to confirm the protective power characterizing these bio-moleculs shows that the presence of both bio natural products. Corrosion rate decreases after four hours after the injection of each product in contemned medium with 10^2 germs/mL of SRB, incubated for 36 to 72 h.

The deferent techniques show the effectiveness of bio natural products for steel protection against MIC, high polarizations resistance are obtained during this study as a function of increasing concentration of bio naturals products tested.

With regard to the electrochemical impedance spectroscopy technique; semi-circles obtained increase for each concentration as from 5 ppm to 30 ppm, satisfactory results were obtained confirming the protective film formed by extracted biomoleculs from green algae marine Ulva Lactuca against corrosion caused by SRB.

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