The Application of Enzymological Techniques for Determination of the Inhibitor Activity of Bio Enzym Extracted from Seawater Bacteria *Pseudomonas aerogenosa* to Fight Biocorrosion of Carbon Steel

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The paper presents a studies for the development of new corrosion inhibitors by biotechnological way applicable to many areas of petroleum industry including, characterization of protective films on carbon steel and biocorrosion inhibition. Our research is oriented towards the isolation of new strains of bacteria from the sea waters of the Mediterranean region that have power (inhibitory and/or bactericidal that blocks the growth of sulfate-reducing bacteria responsible for microbiologically influenced by production of Pyocyanin (1 hydroxyphenazin) or 5-methylphenazin-1-one. Our first objective was to isolate the population of Pseudomonas Aerugenosa on a specific medium, characterization gram, then fermentation in a nutrient broth. The second objective of this study is evaluation of corrosion rate by weight loss method with injecting different dose of the crude enzyme extract CEE containing the methylphenazine (Pyocyanin). An efficacy test was performed on test kit vials containing the specific culture medium with the SRB contaminated with industrial injection water from SONAHESS region. The total disappearance of the SRB is 40% of CEE by blocking their metabolism in the growth phase confirms the efficiency of treatment.

Keywords: Biotechnology, Pseudomonas aeruginosa, fermentation, enzymology, bactericide, biocorrosion, nutrient broth, inhibition, sulfate-reducing bacteria

The microorganisms of the environment are extremely varied in their classes, their families and species. They also differ in their metabolic characteristics (nature of the carbon source, nature of electron acceptor, gene expression) [1]. We will focus in particular bacteria because they represent a major contribution to biocorrosion process and therefore natural attenuation of the deterioration and degradation of materials.

Metal deterioration due to microbial activity is called microbiologically influenced corrosion or corrosion caused by microorganisms (MIC). Because of its economic and environmental importance, (MIC) has been extensively studied over the past five decades and several models have been proposed to explain the mechanisms observed biocorrosion [2, 3].

So the (MIC) refers to the accelerated deterioration of material due to the presence of biofilm on its surface; corrosion rate reaches 1 mm/year, mainly containing sulfate-reducing type of bacteria that play a key role in corrosion anaerobic, producing hydrogen sulfide (H_2S), corrosive metabolite[2-4].

The cost of bio deterioration of metallic materials is estimated at between 5 and 10% of all damage caused by corrosion. To prevent this problem, the Algerian oil industry annually provided approximately 30 million dollars for chemicals such as biocides that are ineffective because of the addiction of bacteria to the product detriment of sulfate-reducing bacteria.

Microscopic biological community more or less complex microorganisms (bacteria, fungi or algae), adhering to each other and to a surface, and characterized by the secretion of an adhesive and protective matrix, is called biofilms. Is generally formed in aqueous medium. Microbial activity in biofilms formed on the surfaces of metal materials can also affect the kinetics of cathodic reactions and/or anode, dramatically altering the chemistry of the protective layers and the physical properties that result. This can lead to an acceleration or inhibition of corrosion [5-7].

So the fight against microorganisms is necessarily different and in order to increase the useful life of the facilities and to significantly reduce maintenance costs, a contribution to the study of biotechnological control techniques applied biocorrosion oil facilities has been the subject of a study presented on this article.

Our experimental work is divided into 5 steps:

- The isolation of bacterial strains on specific environment from our region seawater.
- Identification and characterization of this bacterial population based on different physico-chemical parameters.
- Study of the effect of Pseudomonas Aeruginosa bacteria on the development of sulfate-reducing bacteria (SRB).
- Extraction of crude enzymes extract (CEE) on nutrient broth was made and tested on SRB strains to assess bacterial corrosion rate and efficiency of the molecule in different doses.
- Identification of the nature of the organic functions of the crude enzyme extract (CEE) by Infrared Spectroscopy and proposed bio-enzyme mechanisms action on the phenomenon of microbiologically influenced corrosion in general and particularly on the SRB.

Experimental part

Isolation of bacterial strains from seawater

Sampling of sea water sample was made on a rocky area full of green algae, at Figuier region (caves of Rocher Noir) - Boumerdes - East Algeria, 04/04/2015 at 16h00.

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The physicochemical analyzes of sea water were carried out by: Volumetric Methods (Complexometry, Acidimetry, Argentimetry) Gravimetric methods (precipitation, dry extract) and by Atomic Absorption [8-11].

Sea water is mainly composed of sodium, magnesium, potassium, chloride and sulphates. The average proportions measured in the Mediterranean Sea are given in table 1.

Table1

THE MAIN CONSTITUENT OF SEAWATER (SAMPLE TAKEN ON 04.04.2015 IN THE REGION OF FIGUIER- ROCHER NOIR - BOUMERDES/EAST COAST ALGERIA- MEDITERRANEAN WATER)

Constitue	Symbol	Concentration (g/L)
Chloride	Cl^-	21.40
Sodium	Na^+	11.60
Sulfate	SO_4^{2-}	3.06
Magnesium	Mg^{2+}	1.295
Calcium	Ca^{2+}	0.416
Potassium	K^+	0.390
Bicarbonates	HCO_3^-	0.145
Bromide	Br^-	0.066
Strontium	Sr^{2+}	0.027
Boron	B^{3+}	0.013
Fluoride	F^-	0.001
Total	38.772	

This water is rich in minerals and trace elements considered necessary nutrients for the proliferation of microorganism.

The publication of Klein B. et al, for information rather precisely the seawater composition in terms of salinity, temperature and oxygen content [12]. The parameters are quantized surface. These measures are a good approximation for our study. For the isolation of bacteria, specific environments rich in nutrients necessary for their growth have been prepared using the following composition [13]. Peptone 12.0 g/L - Lactose ($C_{12}H_{22}O_{11}$) - 12.0 g/L - Sucrose ($C_{12}H_{22}O_{11}$) 12.0 g/L- Yeast extract 3.0 g/L - Sodium Chloride (NaCl) 5.0 g/L - Sodium Thiosulfate ($Na_2S_2O_3$) 5.0 g/L - Ammonium Ferric Citrate ($C_6H_{11}FeNO$) 1.5 g/L - Bromothymol Blue 0.065 g/L- Fushin Acid ($C_{20}H_{17}N_3Na_2O_9S_3$) 0.04 g/L - Bacteriological Agar $(C_{12}H_{18}O_9)_n$ 13.5 g/L. *p*H of the medium was adjusted to (7.6 ± 0.2) at 25°C.

Near the flame of a Bunsen burner, the liquid culture medium was poured into the petri dishes. After the medium has solidified, decanted 1mL seawater sample to the surface of the medium, and is incubated at 37°C for 24 to 48 h.

Following the turn of the culture medium of brick red to greenish blue when incubated at 37°C for 48 h (fig.1), we have identified the bacterial population isolated from sea water in a specific environment is *Pseudomonas aerugenosa* [13].

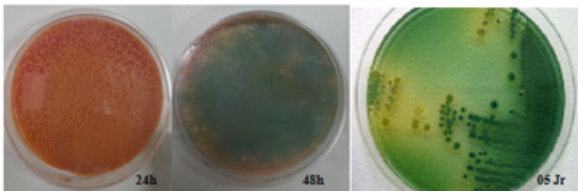


Fig.1. Isolation of *Pseudomonas aerugenosa* bacteria on solid culture medium from seawater (macroscopic identification - Turn the color of the medium during the incubation time)

The color changes from red to greenish blue brick indicates the presence of *Pseudomonas aerugenosa* isolated from sea water. The blue color is specific Pyocyanin (fig. 2), which is a specific pigment produced by the *Pseudomonas aerugenosa* [14].

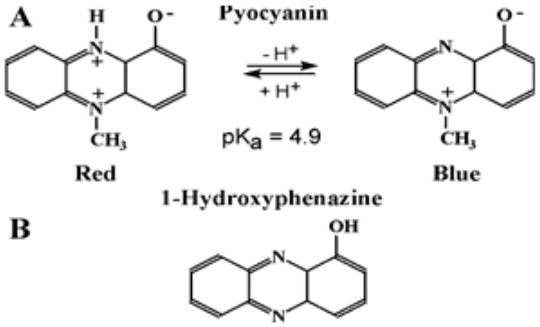


Fig.2. Mechanism of red brick color change to blue in the presence of *Pseudomonas aerugenosa* bacteria [15]

The confirmation of the result was made by microscopic observation of the Gram (fig. 3)

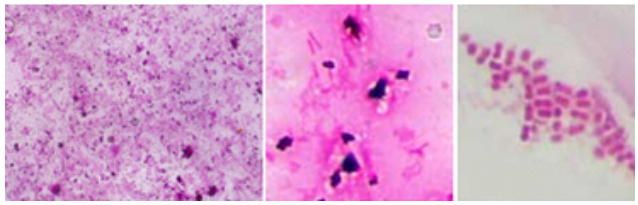


Fig.3. Morphological observation of *Pseudomonas aerugenosa* bacteria optical microscope(Gram negative wall) (X100 Objective).

The bacterial population object is colored pink; this indicates that its wall is of negative Gram [16] and this is consistent with the specifications of the Pseudomonas type bacteria [14]; which are shaped bacillus negative Gram. We conclude that the bacteria isolated from the seawater sample are Pseudomonas aerugenosa.

Isolation of sulphate –reducing bacteria from industrial injection water

Our study was performed on isolated SRB stem from the industrial waters taken from well injection *Agreb#6* of the Field of SONAHESS-SONATRACH-AMERADA-HESS GROUP at HASSI MESSAOUD in south of Algeria (fig. 4).

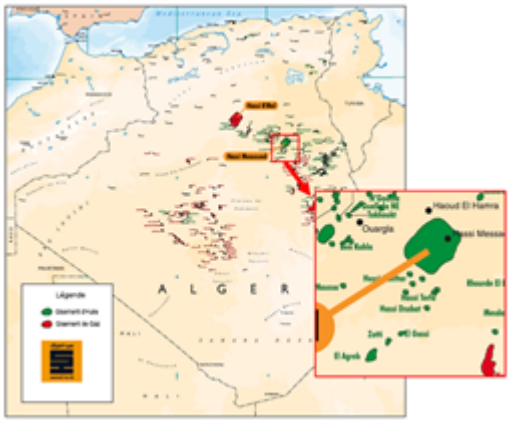


Fig.4. Locationh of the region SONAHESS - Hassi Messaiud - South Algeria

The physico-chemical analysis of injection water have been performed by: Volumetric Methods (Complexometry, Acidimetry, Argentimetry) Gravimetric methods (precipitation, dry extract) and by Atomic Absorption [8-11].

The results of the injection water analysis are summarized in table 2.

Elements	Ca^{2+}	Mg^{2+}	Fe^{2+}	K^{+}	Na^{+}
Concentration (mg/L)	160.3	753.9	traces	294.6	8528.7
Elements	Cl^{-}	HCO_3^{2-}	SO_4^{2-}	Dry extract	Cl^{-}
Concentration (mg/L)	19855.9	184.2	627.97	61740	19855.9

Table 2
PHYSICO-CHEMICAL ANALYSIS OF INJECTION WATER - WELL INJECTION AGREB#6 OIL FIELD OF HASSI MESSAOUD SONAHESS- REGION - SOUTH ALGERIA

For the isolation and/or reactivation of sulfate-reducing bacteria the medium must contain quantitative and qualitative substances required for growth. For this, we used the specific culture medium SRB chemical composition according to Standard API RP 38 [17] the following: Sodium Lactate (60-70%) ($C_3H_5NaO_3$) 4 mL, yeast extract - 0.1g, Ascorbic Acid ($C_6H_8O_6$) - 0.1g, Magnesium Sulfate ($MgSO_4$ .7 H_2O) - 0.2g, Bipotassium Phosphate (K_2HPO_4) Anhydrous - 0.01g $Fe(SO_4)_2$ (NH_4) $6H_2O$ - 0.2 g NaCl - 10 g, distilled water - 1000 mL, the medium *p*H is adjusted to 7.3, the culture medium is distributed in the penicillin bottles at 9 mL per vial. The vials are sealed with rubber stoppers and then capped with a manual seamer with disposable metal lids. The oxygen content in the vials was removed by sparging with nitrogen [17].

The vials are sterilized by autoclaving at 1 bar pressure for 15 min (sterilization with steam under pressure) at 120°C. For the highlighting of sulfate-reducing bacteria in industrial water we performed by successive dilution of test kit; 18 penicillin vials divided into three sets of six (06) assembled and labeled vials.

This series is called *Dilution Test Kit* in the following steps: using a syringe, we collected 1mL of industrial water and injected into the first penicillin bottle, after shaking and the using the same syringe was taken 1 mL of the first penicillin bottle and injected into the second bottle of the same series, after agitation and using the same syringe, we proceeded as before dilutions the third to the sixth bottle. These are incubated at 37°C for 28 days [17].

Results and discussions

Influence of physicochemical parameters on pseudomonas aerugenosa bacterial activity

We studied the Influence of physico-chemical parameters on bacterial growth, metabolism [18] and the activity of the latter by measuring Optical Density (O.D) using a spectrophotometer, in different conditions of: temperature, *p*H, salinity, incubation time and the presence of SRB.

Temperature influence

Each type of bacteria has a specific temperature for proliferation [19]. Our bacterial strain is a clear sensitivity to the temperature change; the results are recorded in table 3.

O.D is minimal for low temperatures (-4 to 10°C) and at high temperatures (100°C), against by our bacterial strain shows good resistance to temperatures ranging from 22 at 60°C and its activity is maximal at 37 to 40°C.

Influence of pH

The results of the optical density recorded for the different *p*H environments are summarized in table 4.

From the curve obtained, which shows the influence of *p*H medium on the optical density of Pseudomonas Aeruginosa, we find that the maximum values of the O.D or (0.430% and 0.415%) are recorded at *p*H = 8.5 and 9.2 respectively, thus neutralizing the culture medium promotes the development of our bacterial strain.

Temperature T (°C)	- 4	5	22	37	40	50	60	100
optical density O.D (%)	0.071	0.218	0.531	0.610	0.602	0.530	0.402	0.175

Table 3
INFLUENCE OF TEMPERATURE ON BACTERIAL ACTIVITY OF *Pseudomonas aerugenosa*

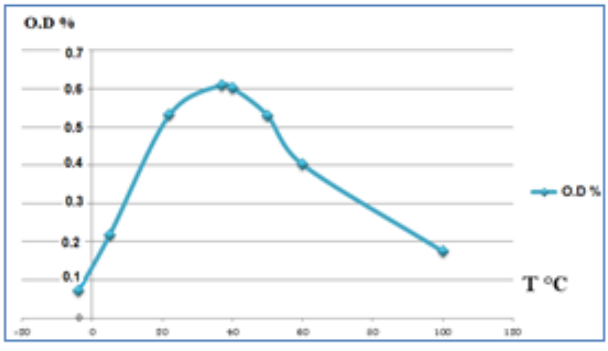


Fig.5. Changes in optical density depending on the temperature of incubation of *Pseudomonas aeruginosa* strains.

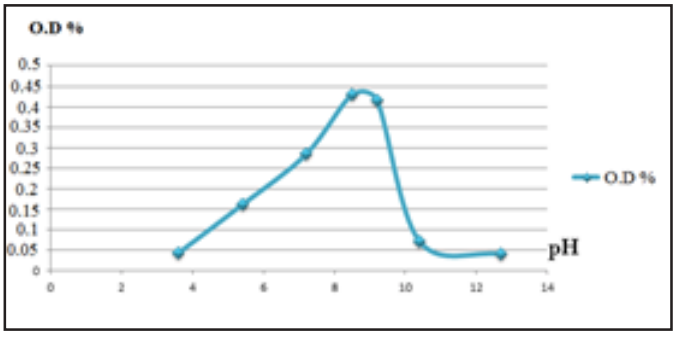


Fig.6. Evolution of the optical density of *Pseudomonas aerugenosa* as a function of *p*H of medium

pH of medium	3.6	5.4	7.2	8.5	9.2	10.4	12.7
O.D (%)	0.043	0.161	0.284	0.43	0.415	0.071	0.04

Table 4
INFLUENCE OF *p*H ON THE ANTIBACTERIAL ACTIVITY OF *Pseudomonas aerugenosa*

NaCl Concentration (g/L)	5	10	35	60	150
O.D (%)	0.151	0.214	0.319	0.156	0.152

Table 5
INFLUENCE OF SALINITY ON THE BACTERIAL ACTIVITY OF *Pseudomonas aerugenosa*

Table 6
INFLUENCE OF INCUBATION TIME ON THE BACTERIAL ACTIVITY OF PSEUDOMONAS AERUGENOSA

Time (Days)	1	2	3	4	5	6	7	8	9	10
O.D (%)	0.085	0.125	0.229	0.53	0.61	0.602	0.581	0.497	0.305	0.22

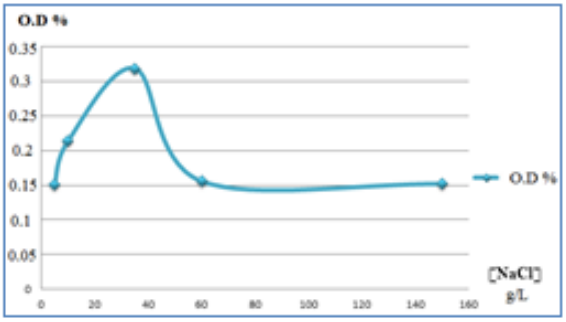


Fig.7. Evolution of the optical density as a function of salinity of the medium

Influence of salinity

The results of this study are recorded in table 5

Following the curve shown in figure 7, we see that the optical density grows exponentially as a function of the salinity of the medium until it reaches an optimum at a NaCl concentration of 35 g/L, beyond this concentration, it decreases; this is due to the reduction of the bacterial activity of *Pseudomonas aerugenosa*.

Influence of incubation time

The curve given in figure 8 shows the evolution of the bacterial activity in function of incubation time for 10 days. We find that O.D increases over time to a maximum value given on the 6 and 7 days; which explains that growth has a maximum speed in this phase *exponential growth phase*, then it decreases until the last day when we have the *decline stage*. The curve obtained is identical to that of bacterial growth [20]. It represents the various phases of growth of microorganisms.

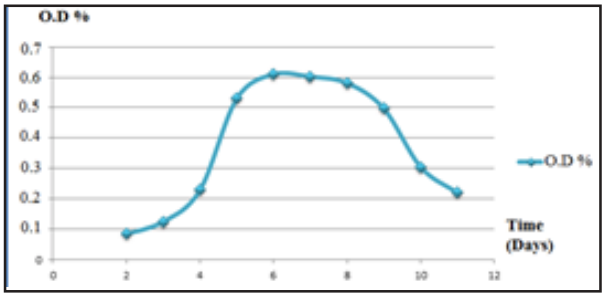


Fig.8. Evolution of the optical density as a function of incubation time *Pseudomonas aerugenosa*

Table 7
INFLUENCE OF THE CONCENTRATION OF SRB ON *Pseudomonas aerugenosa* BACTERIAL ACTIVITY

SRB concentration (germ/mL)	10	10^2	10^3	10^4
O.D (%)	0.288	0.377	0.202	0.150

Influence of the concentration of SRB on the activity of Pseudomonas aerugenosa

The results of the evolution of the optical density versus the concentration of SRB, shown in figure 9, indicate that the optical density represents a peak at 10^2 germs/mL concentration of SRB, outside this concentration it decreases, which explains that the bacterial activity of Pseudomonas is maximal at 10^2 germs/mL concentration of SRB, then it decreases gradually as the concentration of SRB increases.

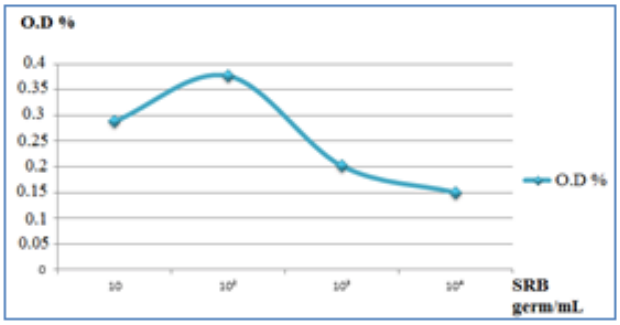


Fig.9. Evolution of the optical density versus the concentration of SRB

Extracting the enzymes produced by pseudomonas aerugenosa

Preparation of nutrient broth

For the fermentation of Pseudomonas Aerugenosa we prepared a nutriment broth for cultivation of sprouts object, the chemical composition of the culture medium is as follows: Peptone (10 g/L); Sodium Chloride NaCl (0.5g/L); Beef Extract (10 g/L). The *p*H of the medium is adjusted to 7, and then sterilized for 15 min at 120°C [21].

Fermentation in nutrient broth

All bacterial suspensions obtained in boxes kneaded from the marine bacterium have been in this nutrient broth and allowed to incubate for 48 to 96 h. We noticed a turbidity of the medium (fig. 10) and a *p*H shift from 7 to 8.15.

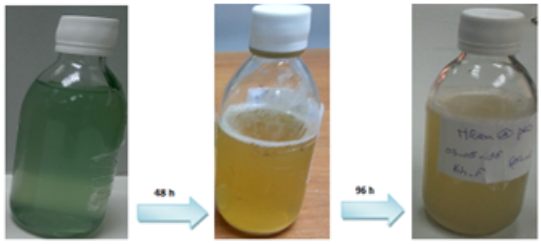


Fig.10. Appearance of change nutrient broth after 48 to 96 h incubation at 37°C

Extraction by centrifugation

After 96 h of incubation, we performed centrifugation nutrient broth for 4 h to extract part of Pseudomonas Aeruginosa enzyme. To a volume of 100 mL broth, we extract 30 mL volume of crude enzyme extract (CEE) making a yield of 30% thereof. After centrifugation we obtained two phases; the pellet and supernatant (fig. 11) to identify the phase that contains our CEE, we tested the effectiveness of two phases of the SRB, the pellet produced a positive result which showed that the extract crude enzyme was in this phase (base).

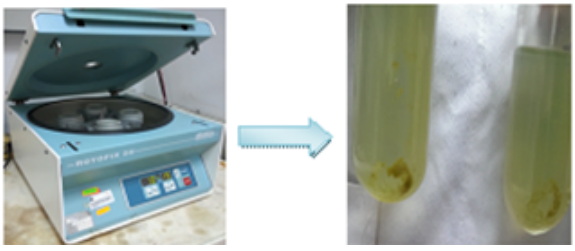


Fig.11. Extraction of crude enzyme extract by centrifugation - Obtaining two phases (Pellet and supernatant)

Evaluation of bacterial corrosion

Evaluation of the corrosion rate by weight loss method

The corrosion rate is determined by evaluating the weight loss of steel coupons immersed in an injection water solution with presence of different concentrations of the product (either bacterial suspensions of Pseudomonas Aeruginosa strains BS or crude enzyme extract CEE).

After 15 days of exposure, the coupons were removed and cleaned then weighed to determine the corrosion rate by gravimetric and determine the efficiency at different B.S concentration; the results are given in tables 8 and 9.

Treatment with the bacterial suspension [B.S]

From the curve shown in figure 12 the evaluation of the corrosion rate by the weight loss method depending on the concentration of bacterial suspension of Pseudomonas Aerugenosa.

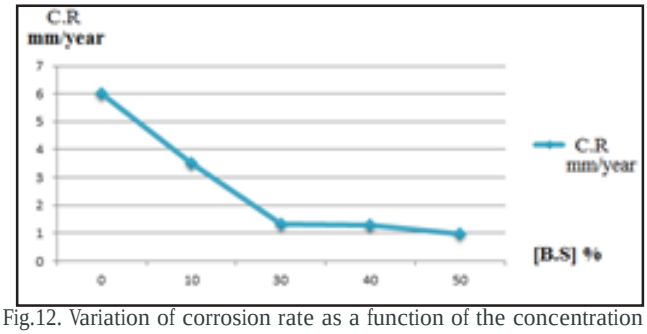


Fig.12. Variation of corrosion rate as a function of the concentration of bacterial suspension

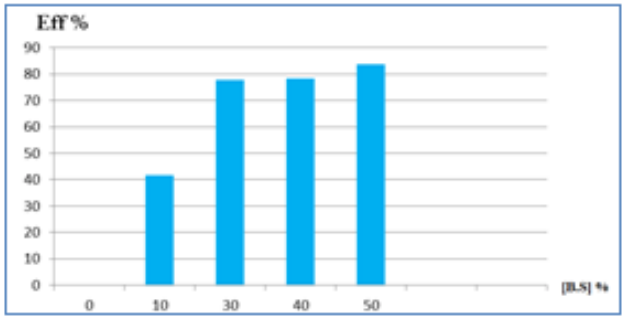


Fig.13. Histograms of the efficiency of the bacterial suspension at different concentrations

We see that the corrosion rate decreases as the concentration of the bacterial suspension of Pseudomonas increases in the medium (injection water solution), the blank corrosion rate (without treatment) was 6.018 mm/year, after injection 30% of B.S it reached 1.3325 mm/year; at 50% concentration thereof, the corrosion rate decreases to 0.9787 mm/year.

This curve is translated by the histograms shown in figure 13 which represents the efficiency of treatment for different doses of bacterium Pseudomonas Aerugenosa. We note that for 40% of bacterial suspension concentration, the efficiency is close to 80% (equivalent to 78.47%) and 50% of S.B it reaches 83.73%. The protective power given by Pseudomonas strains is tolerable, but not enough to fight against the growth of SRB on site, why we proceeded to treatment with the CEE to achieve a satisfactory protection rate that exceeds 90% efficiency of required treatment on oil sites.

Treatment with crude enzyme extract (CEE)

The curve shown in figure 14 represented the evaluation of corrosion rate by the weight loss method to the different concentration of CEE show that the corrosion rate decreases gradually as the concentration of CEE increases, corrosion rate of the Blank coupon was 8.0775 mm/year, and after injection of 1% of CEE, it reaches 0.162 mm/year, then it decreases to 0.0029 mm/year at 50 % by CEE. According to the histograms (fig. 15) representing the

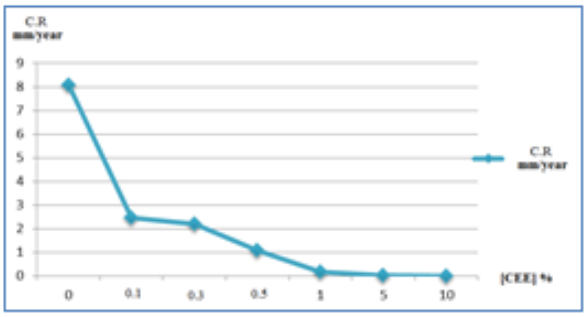


Fig.14. Change in corrosion rate according to different concentrations of crude enzyme extract (CEE)

[B.S] (%)	W_i (g)	W_f (g)	ΔW (g)	A (cm^2)	d (g/cm^3)	C.R (mm/year)	Efficiency (%)
0	22.6046	18.5959	4.0117	2.08	7.8	6.018	-
10	16.0125	13.9875	2.025	1.8	7.8	3.5101	41.67
30	20.0228	19.0065	1.0163	2.38	7.8	1.3325	77.85
40	21.7898	20.9657	0.8241	1.98	7.8	1.2986	78.47
50	24.8655	24.2444	0.6211	1.98	7.8	0.9787	83.73

Table 8
EVALUATION OF THE CORROSION RATE DEPENDS ON THE CONCENTRATION OF THE BACTERIAL SUSPENSION OF *Pseudomonas aerugenosa*

[CEE] (%)	W_i (g)	W_f (g)	ΔW (g)	A (cm^2)	d (g/cm^3)	C.R (mm/year)	Efficiency (%)
0	14.2398	10.5376	3.7021	1.43	7.8	8.0775	/
0.1	14.02	12.9109	1.1091	1.4	7.8	2.4717	69.40
0.3	13.1238	12.3018	0.8220	1.17	7.8	2.192	72.86
0.5	14.1787	13.6987	0.4800	1.4	7.8	1.0697	86.75
1	14.6458	14.5658	0.0800	1.54	7.8	0.162	97.99
5	12.7478	12.7394	0.0084	1.08	7.8	0.0243	99.69
10	15.3853	15.3824	0.0029	1.65	7.8	0.0054	99.93

Table 9
EVALUATION OF CORROSION RATE DEPENDS ON THE CONCENTRATION OF CEE

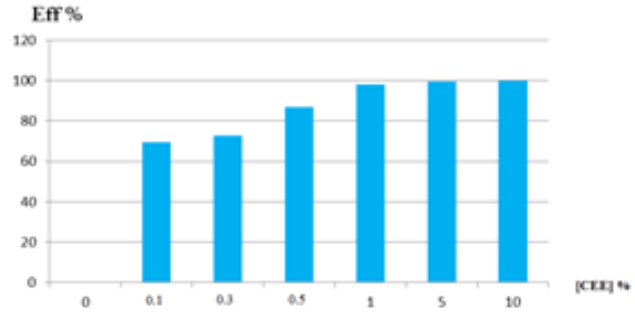


Fig.15. Histograms efficiency of crude enzyme extract at different concentrations

efficiency of CEE in different doses, we find that the concentrations (1, 5, 10%) of CEE provided satisfactory efficiencies (97.99 , 99.69 ; 99.93 %). Therefore, treatment with CEE has given a satisfactory result to protect carbon steel against corrosion caused by SRB. This product removed all the SRB in injection water.

To determine the nature and the organic function of Crude enzyme extract produced by Pseudomonas Aerogenosa, we analyzed our CEE by infrared spectroscopy technique.

Microscopic observation of steel coupons with and without treatment.

Pictures taken by the SEM clearly show the difference between the steel surface exposed to different electrolytes, figure 16a shows a clear appearance; while Figure.16.b shows pitting on the entire surface of the steel placed as colonies, confirming their training under the biofilm of SRB, figure 16c shows an aspect more or less clear to the surface appearance less pitting in small diameters, figure16d shows a protective film on the surface of the steel and total absence of pitting. Confirming the efficiency of treatment with the CEE which prevented biofilm formation of SRB to carbon steel surface.

According to the results of evaluating the corrosion rate by method of weight loss, we proposed two hypotheses:

- We can consider the concentration of CEE as a bactericide, the SRB adsorb bactericidal, it blocks the process of bacterial metabolism at a very specific time (latency period) for the high dose after a given contact time, the bacterium dies. [15]

- The CEE can be considered a bacteriostatic product, since at low concentrations, the bacteria blocked the capacity itself exchanges with the outside until it is no more product. So there, blocking the metabolism but the

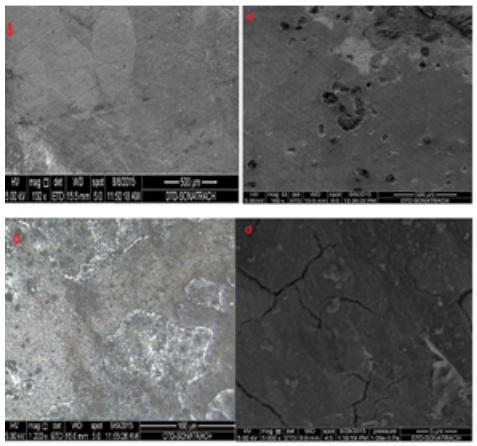


Fig.16.SEM of the carbon steel surface API5LX60 shade different electrolyte immersed in a,b,c and d. a)Non-contaminated injection water, b) Injection water contaminated by SRB c) Injection water contaminated by BSR and treated with 10% B.S d) Injection water contaminated by SRB and treated with 50% of CEE

bacteria is not killed. Once the cause of product proliferation again, which explains the high rate of corrosion in the presence of low concentration of the bacterial suspension of Pseudomonas (B.S).

Efficacy test of the extract crude enzyme for 28 days

Daily readings penicillin vials were performed during the 28 day study, these readings were performed in the same manner as those of the test kit, and summarized in table10

According to the readings of monitoring, the efficiency treatment of SRB contamination and the CEE injection at different doses, we find that:

- Untreated vials recorded contamination of 10^4 germs/mL from day 10 of incubation at 37°C.

- For treated flasks to concentrations of CEE from 0.1 to 20%, contamination persists and reaches 10^4 germs/mL from day 10 of incubation.

- For treated flasks to 30% of CEE, no contamination was recorded for 14 days but, 10 germs/mL of SRB concentration appears on the 15th day of incubation.

- For high concentration at 40% and 50% of CEE, the concentration of SRB is zero during the 28 days of incubation.

This can be explained by the mechanism action of inhibitory molecules of CEE blocking the active site in the SRB but in sufficient doses, which was confirmed by the results in table 10.

Time (Days)

[CEE] %	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
0	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
0.1	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
0.3	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
0.5	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
1	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
5	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
10	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
15	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
20	0	10	10	10	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}	10^{9}
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10
40	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
50	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

Table10
FOLLOWED THE EFFICIENCY OF CRUDE ENZYME EXTRACT

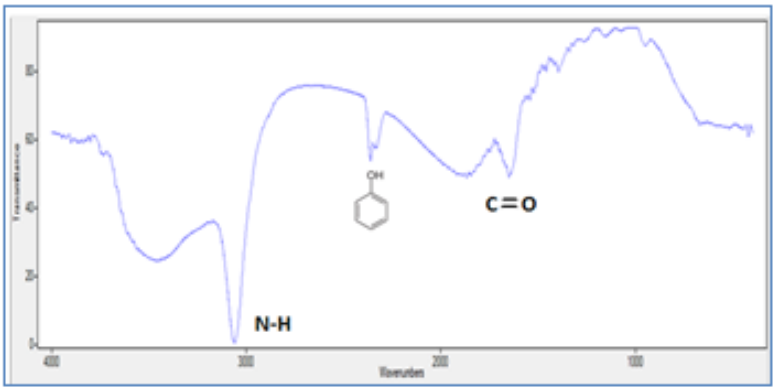


Fig.17. Infrared spectrum of the enzyme protein (CEE)

Identification of the enzyme protein derived from pseudomonas aerugenosa bacteria

-An infrared spectroscopic analysis was performed to identify the organic functions present in the crude enzyme extracted. The results of this analysis are given in the spectrum of figure 17

-The spectrum shows two medium intensity absorption bands around 3400 cm^{-1} and 3300 cm^{-1}, this indicates the presence of the N-H group.

- The spectrum indicates the presence of the amine function between 3330 cm^{-1} and 3250 cm^{-1}.

- The spectrum showed mean intensity absorption bands at strong in the area 1650 cm^{-1} and 1450 cm^{-1}, this indicates the presence of the double bond to alkenes.

- The spectrum has an absorption peak very fine average intensity to 2250 cm^{-1} indicating the presence of the phenol ring.

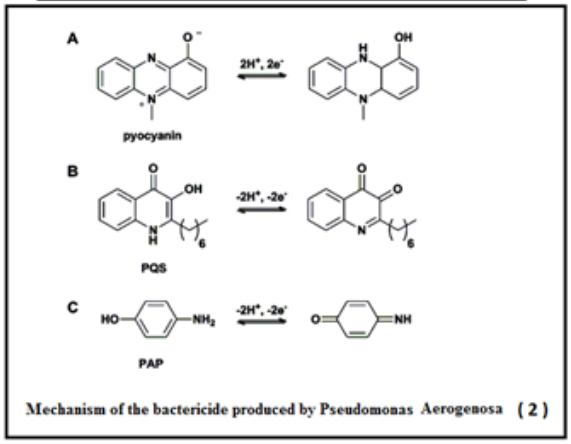
The spectrum shows the molecule methyl phenazine-1-one or pyocyanin, the molar mass of 210.33 molecule and chemical formula $C_{13}H_{10}N_2O$ responsible for blocking the growth of SRB by the use of hydrogen, the SRB need to produce the hydrogenase enzyme responsible for their growth [15].

Proposed mechanism of action of Pseudomonas aerugenosa

When material (carbon steel) is immersed in an electrolyte (conductive solution or an industrial water injection rich in chloride), oxidation-reduction reactions of the metal represented by the mechanism (1) will take place. After contamination of the environment by the sulfate-reducing bacteria, these microorganisms reduce sulfates water sulfide and produce H_2S according to the mechanism (3). The sum of reactions (1) and (3) causes

$$2H^+ + 2e^- = H_2$$
$$O_2 + 2H_2O + 4e^- = 4OH^-$$
$$Fe = Fe^{2+} + 2e^-$$
$$Fe + 2H_2O = Fe(OH)_2 + 2H^+ + 2e^-$$
$$Fe^{2+} + 2H_2O = Fe(OH)_2 + 2H^+$$

Mechanism of corrosion of a carbon steel in an aqueous medium (1)



Mechanism of the bactericide produced by Pseudomonas Aerogenosa (2)

$$SO_4^{2-} + 10H^+ + 8e^- = H_2S + 4H_2O$$
$$S^0 + 2H^+ + 2e^- = H_2S$$

SRB corrosion mecanism (3)

Fig.18. Pyocyanin Mechanism of Action for the fight against corrosion induced by SRB

biocorrosion and form the product of the FeS corrosion, but the presence of the bacteria Pseudomonas Aerugenosa or the crude enzyme extract of these bacteria isolated from sea water, blocks the mechanism (3) by their product of the mechanism (2) which is Pyocyanin, known by its inhibitory power to other types of bacteria [15] and prevents the growth of SRB by using the hydrogen they will need to produce hydrogenase responsible for bacterial growth enzyme.

Conclusions

CIM in anaerobic medium is a very complex process. While the overall mechanism is known and methods to fight against the phenomenon are effective, the fact remains that the research of biotechnological control technologies remains a means in development.

We are interested to study bacterial corrosion and its fight to protect oil installations. we studied this type of corrosion in order to develop an antibiocorrosion biotechnologically treatment based on the study of enzymological assay techniques an enzyme extracted from the bacteria isolated from sea water in the region of Figuier Boumerdes and follow their effect on sulphate reducing bacteria responsible for microbiologically influenced corrosion, isolated from injection water of SONAHESS SONATRACH group oil field in Hassi Messaoud- Algeria.

Chemical analyses of the seawater samples and industrial water, have shown that they are rich in nutriments and trace elements necessary for development of microorganisms. Isolation of strains from sea water on specific environment and characterization of Gram, have confirmed the presence of Pseudomonas Aerugenosa population.

The study of the influence of physico-chemical parameters on Pseudomonas bacterial activity by measuring optical density allowed us to achieve a data sheet for this population. Therefore the development of Pseudomonas Aerugenosa is done at temperature ranging from 37 to 40°C for 24 to 48 h of incubation, their presence causes a shift of the red brick culture medium to the blue at a medium *p*H between [8.5 to 9.2], at a salinity of 35 g/l of NaCl and is resistant to temperatures ranging from 37 to 40°C.

To determine their mechanism of action on the SRB, a study of the evolution of corrosion rate by weight loss of steel API 5L X70 shade of carbon for a period of two (02) weeks, has confirmed their power inhibit other bacteria by blocking their growth metabolism.

To identify the type of biomolecule and/or functions responsible for the inhibition of bioccorosion, fermentation of Pseudomonas Aerugenosa on nutrient broth allowed us to extract the crude enzyme and test on a contaminated water and steel, a greater than 95% efficiency is 99% is obtained from a dose of 10% of CEE making a corrosion rate (0.0054 mm/year) was obtained.

The infrared spectroscopic analysis of CEE has confirmed the presence of an amine function, cyclic molecules, phenol, double bond, the product spectrum belongs to Pseudomonas which is Pyocyanin (named by the IUPAC 5-methyl phenazin-1-one) known by its inhibitory power/bactericidal other type of bacteria.

Based on the results we recommend confirming these proposals by a thorough study of electrochemical techniques for evaluating the bacterial corrosion rate caused by SRB in the presence of Pseudomonas Aerugenosa called this population and test its crude enzyme extract different doses.

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