INTERRELATIONSHIP BETWEEN CATHODIC PROTECTION AND MICROBIOLOGICALLY INFLUENCED CORROSION IN MARINE ENVIRONMENT: BRIEF-REVIEW AND PROSPECTS

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ABSTRACT

Cathodic protection is widely used to prevent corrosion of steel constructions exposed to seawater. This protection causes a calcareous deposit as well as a biofilm formation. The Sulphate-Reducing Bacteria (SRB) and Thiosulphate-Reducing Bacteria (TRB) are involved in the steel biodegradation phenomena in marine environment. The surface colonization by these species under cathodic protection are described particularly for the TRB and with immersion periods of over 30 days. After a brief-review, preliminary studies explain the first results of our investigation in natural seawater and carbon steel during 4 to 8 weeks experiment. We have made experiments with natural strains of SRB/TRB, with monoculture of *Desulfovibrio halophilus* (SRB) and *Dethiosulfovibrio peptidovorans* (TRB), with potential a Cathodic Protection (CP) of –900 mV/SCE and –1000 mV/SCE and without CP. These results seem indicate that the effects of CP on the sulphide-producing bacteria metabolism is the reduction of their corrosive activity.

INTRODUCTION

Cathodic protection (CP), using sacrificial anodes or impressed current, is widely used to prevent corrosion of steel constructions exposed to seawater. This provides electrons to the metal surface and allows only the cathodic oxygen reduction reaction to occur. There is no possible metal iron dissolution in that sense thatcorrosion is prevented. CP leads to an increase of interface pH [1]. This alkalinity causes the precipitation of calcium and magnesium compounds to form a calcareous scales. These calcareous deposits are an important factor in the protection of metal. They can provide a physical barrier to general corrosion attack, reduce the flux of dissolved oxygen from the bulk solution towards the metal surface, reduce the current density required to maintain a given potential, and consequently decrease the cost of CP [2][3].
In natural marine environment, micro-organisms colonize surfaces and form a biofilm on all exposed materials, including cathodically protected surfaces. In aerated seawater, a cathodic polarization of –800 mV/SCE is normally sufficient to prevent steel corrosion. The action of the biofilm enhances both the thermodynamics and kinetics of the cathodic reaction of oxygen reduction. The main consequence of the biofilm on the electrochemical phenomenon is an increase in the current density required to polarize and protect the metal [1][3][4][5][6].
In anaerobic conditions associated with marine sediments or under large attached macrofouling deposits, this potential must be lowered (about –900 mV/ECS) due to the presence and activities of micro-organisms such as sulphate-reducing bacteria (SRB) and thiosulphate-reducing bacteria (TRB) [2]. SRB and TRB are frequently associated

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with corrosion in marine environment in industrial installations such as pipelines, storage tanks, jetties, heat exchangers or cargo/ballast compartments.
The interrelationship between SRB/TRB biofilm formation and CP efficiency on carbon steel is very important to estimate the effectiveness of this protection system, to understand the effects on bacterial metabolism and consequently the risk for harbour structures.
This paper is split into 2 parts: the first part is a short-review of interrelationship between CP on carbon steel and marine biofouling, the second part explains some results obtained from preliminary studies in natural seawater and medium for pure cultures, with and without CP.

BRIEF-REVIEW

Biofilm and calcareous deposits

Interface pH plays a major role in calcareous deposition and biofilm formation. The interface pH depends on water chemistry, bacterial species and applied current density. CP increases the pH whereas anaerobic biofilm decreases it [2][3]. Organic acids produced by bacterial activities can modify the interface pH and hence change the kinetics, the constituents, and the structural stability of the deposit. Several authors showed modifications in the amount of calcium and magnesium and deposit stability. On the one hand, organic acids produced by many marine bacteria such as metabolites can change the kinetics of formation of the protective layer of calcium and magnesium salts formed under CP or affect the stability of such a deposit [1][2][4][6][7]. The presence of extracellular polymeric substances produced by bacteria seems to improve the stability and density of calcareous deposit [8][9]. On the other hand, the presence of cations such as Ca^{2+} can favour the bacterial attachment because the extracellular surface charges are negative. These divalent cations could act as bounding to stabilize the extracellular polymeric substances involved in the bacterial attachment.

Cathodic protection and bacterial attachment

CP seems to be effective for controlling the growth of aerobic bacteria on carbon structures immersed in seawater whereas it could improve the growth of anaerobic bacteria such as SRB and TRB.
In 1992 Edyvean et al [1] showed that CP reduces the settlement and reproduction of aerobic bacteria during the early stages of exposure and that the degree of this reduction depends on the current density. Identically, De Mele [10] and Videla et al [4] showed that CP inhibits bacterial adhesion and reproduction in the early stages of the aerobic bacteria biofilm formation.
CP affects adsorption processes for aerobic bacteria [1][4][10]. But when a steady state in the biofilm formation process is achieved, the effect of CP is not relevant. The cathodic hydrogen production and the oxygen reduction probably limit the aerobic bacteria attachment. After some weeks or some months, the thickness of the deposit/biofilm complex probably changes local electrochemical characteristics of the interface, thus allowing bacteria colonization.
In the case of anaerobic bacteria (such as SRB and TRB), CP improves the biofilm formation by favouring anaerobic conditions under the deposit [1][5][6][7][11]. In marine sediments as well as under a biofilm, cathodically produced hydrogen can stimulate the growth of bacteria containing hydrogenase such as SRB [2]. However, the enzymatic and metabolic bacteria activities with CP are not clearly understood.

In 1981, Ulanovskii & Ledenev showed that CP, with or without of SRB, decreases the corrosion attack by a factor of 8 to 9 [12]. More recently, Videla et al [4] observed localised corrosion (pitting) on carbon steel with and without CP in presence of SRB (*Desulfovibrio vulgaris* and *Desulfovibrio desulfuricans*). These small pits are frequently covered by bacteria. In 1992, Dexter and Lin [3] showed also pits using a scanning electron microscopy and concluded that SRB metabolits induce the attack of steel. This effect is not eliminated by CP. Nevertheless, the presence of bacteria on or near pits is not sufficient to explain microbiologically influenced corrosion (MIC).
In presence of SRB, FeS is frequently detected in the deposit (Fig 1). It therefore follows that under discontinuous CP application (for example, due to a temporary system failure), SRB growth could cause severe damage to structures [6][9][13]. It is also possible that hydrogen production can lead to extensive bubble formation, thereby contributing to disruptions in the calcareous deposits.

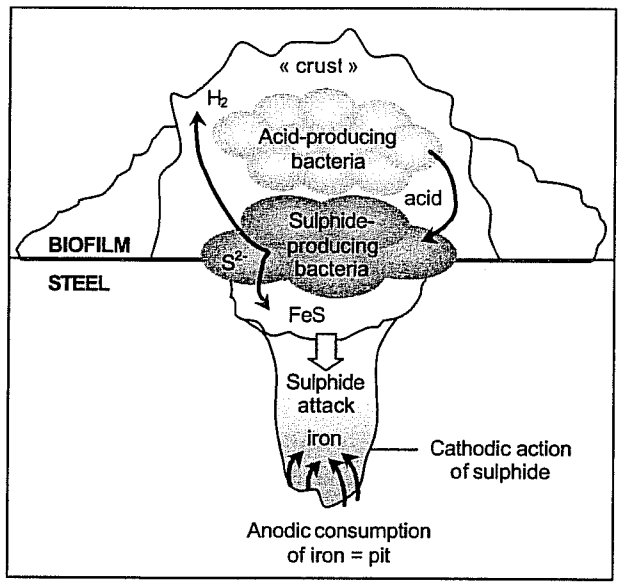

Fig. 1 : example of microbiologically influenced corrosion by sulfide-producing bacteria on carbon steel.

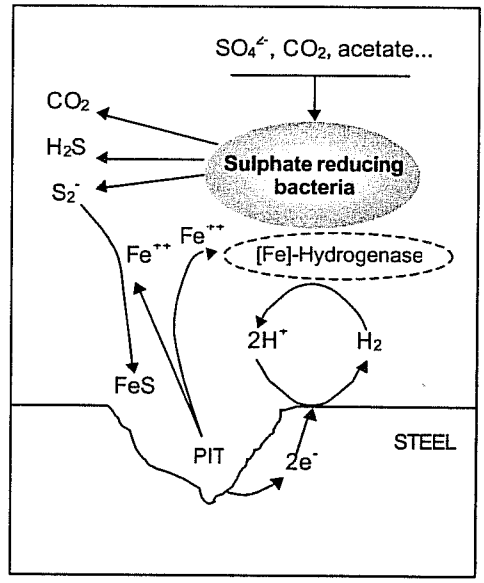

Fig. 2 : possible mechanisms to explain microbiollogically influenced corrosion by cathodic depolarization with [Fe] hydrogenase.

Many authors have agreed with the efficiency of CP in marine environment with bacteria, but we do not know why the corrosive effects of these bacteria (SRB or TRB) are limited with CP.
One hypothesis is that of a metabolic or enzymatic modification of bacteria. One of the main enzymes studied in the MIC phenomena is hydrogenases (fig. 2). Several intracellular or periplasmic hydrogenases have been identified and they can play a major role in steel biodegradation [14][15][16].
The first theory of cathodic depolarisation explains the role of SRB in anaerobic microbiologically influenced corrosion of steels [17]. SRB, which consume hydrogen in their metabolism, could shift the equilibrium between proton and hydrogen in solution and consequently increase the rate of proton reduction that would result in enhancing the oxidation of iron [16].
Some authors suggested that corrosion is a kinetically controlled process and not an equilibrated process. Consequently, the corrosion rate cannot be influenced by the consumption of hydrogen, which is the end product [18]. Nevertheless, Chatelus et al [19], Bryant and Laishley [20] have thought that hydrogenase localised inside SRB, and

exocellular hydrogenase entrapped in the biofilm, could induce a cathodic depolarisation phenomenon.
Moreover, there is no correlation between the number of SRB detected in a given corrosion site and the degree or severity of corrosion [21]. There is another evidence suggesting that the activity of the hydrogenase plays an important role in the corrosion process (Fig 2). Bryant et al (1993) [14] showed that the SRB periplasmic [Fe] hydrogenase is regulated by the Fe^{2+} availability. Since different micro-organisms have a different range of hydrogenase activities, it is possible that a small number of an SRB with a very active hydrogenase in search of iron may be more corrosive than a much larger number of iron-replete SRB with considerably less-active hydrogenase [14].
Finally, the iron dissolution with CP is low. Consequently the [Fe] hydrogenase activity decreases and it is possible that the phenomenon limits the corrosive activities of the SRB.

PRELIMINARY STUDIES

Many research teams have studied the interrelationship between biofouling and CP system. Several studies deal with the bacterial attachment and surface colonization but only few investigate the effect of CP on bacterial metabolism, with TRB, using natural seawater during some weeks. This is the reason why our laboratory has chosen to investigate furthers these experimental conditions. The results presented in this paper are only samples of the research conducted in our laboratory.

MATERIALS AND METHODS

Experimental material and cathodic protection

Carbon steel samples were cathodically polarised to –900 and –1000 mV against a standard calomel electrode (SCE), using a potentiostat (Tacussel® PRT 10-0.5) and platinum counter electrode. Test electrodes were cut from steel sheet pil used in Le Havre Harbour. The nominal composition of the alloy (%, w/w) was 0.178 C, 0.46 Si, 1.41 Mn, 0.01 S, 0.03 P, 0.04 Ni, 0.02 Cr, 0.01 Mo. "Rectangular" electrodes (15 mm x 15 mm x 5 mm) were abraded through 1000 grit carbide metallurgical paper, washed with water and degreased with acetone. The edges of each electrode were masked with an "insulating" resin (Combisub® T150) to expose an area of 2.25 cm^2 to the electrolyte. All electrodes received a final rinse in ethanol before use.
The relationship between bacteria, calcareous deposit and steel surface was examined by Scanning Electron Microscopy (SEM) Cambridge® 240. SEM samples were prepared by rinsing in filtered 30 $g.l^{-1}$ NaCl solution before sputter coated (Au). This observation is completed with Energy Dispersive Spectrometry analysis (EDS). We examined the surface coupons but also the sliced samples to observe the steel relief characteristics such as pits.
The close-system "bioreactors" used have a volume between 1.3 to 8 liters, the agitation is caused by an addition of [N_2]. They are sterilised with ethanol, UV-rays or autoclave (121°C during 15 min).
Two kinds of biofilms were used in this work.

Natural bacterial strains experiment

The first type of biofilm was made of natural population of SRB and TRB. Natural seawater was collected in Le Havre harbour (Port Autonome du Havre), the growth of natural SRB and TRB strain is favoured by anaerobic conditions (N_2 addition), nutrients

compounds (sodium sulphate, sodium thiosulphate and yeast extract). Metal specimens were exposed to natural SRB and TRB cultures for 5, 15, 30 and 60 days.

Monoculture experiment

The second type of biofilm used in these experiments was made of monoculture of SRB (*Desulfovibrio halophilus*, strain DSMZ 5663) and TRB (*Dethiosulfovibrio peptidovorans*, strain DSMZ 11002). The pure culture was innoculated into 1300 ml of filtred natural seawater (0.2 µm Millipore® filter). The Magot's medium [22] is used for TRB culture, and the Starkey's medium [23] is used for SRB culture. These mediums were made of natural seawater collected in Le Havre Harbour (France). This seawater was passed through a 0.2 µm membrane filter before use.
Metal specimens were exposed to *Desulfovibrio halophilus* and *Dethiosulfovibrio peptidovorans* cultures for 5, 15 and 30 days.
During the test periods, the average temperature was 30°C ± 2, the dissolved oxygen was 0.10 to 2.61 ppm, the pH was 8.1 to 8.6, and the salinity was 30 to 35 parts per thousand (ppt).

Microbiological analyses

The bacterial densities of the solution in the biofilm were determined by epifluorescence microscopy with acridine orange staining. Specimens were examined at 1000x magnification under oil immersion in an Olympus BH2 microscope with a vertical fluorescence illuminator. The quantities of bacteria were counted in 20 randomly selected areas and averaged.
The quantities of viable SRB present in the biofilms were enumerated using the serial dilution method with three replicates and standard plating method. Samples were washed in sterile synthetic seawater and thereafter scraped with a scalpel. The biofilms were removed from the specimen using a short period of sonication (35 KHz during 5 minutes). The enumeration was carried out in Starkey's medium, Magot's medium and Plate Count Agar (PCA).

RESULTS AND DISCUSSION

Natural strains of SRB and TRB

Fig. 3 shows the results of mixed natural populations enumeration of anaerobic bacteria (a majority of SRB and TRB) settled on carbon steel under various levels of CP. It can be noticed that the number of attached bacteria increases quicker under CP than without CP. However, after 30 days of immersion the cell number per cm^2 is similar for all experimental conditions. It seems that the CP improves or accelerates the attachment process of anaerobic bacteria during the first days of exposure in natural seawater. This result is in agreement with the literature [1][6][11].

During these experimentations, a lot of TRB were observed during the enumeration . As it was shown by Garcia et al. [24] SRB could grown in TRB medium and therefore increase the enumeration results. It is necessary to develop a new microbiological methods for the identification, the molecular characterisation and the enumeration of sulphide-producing bacteria.

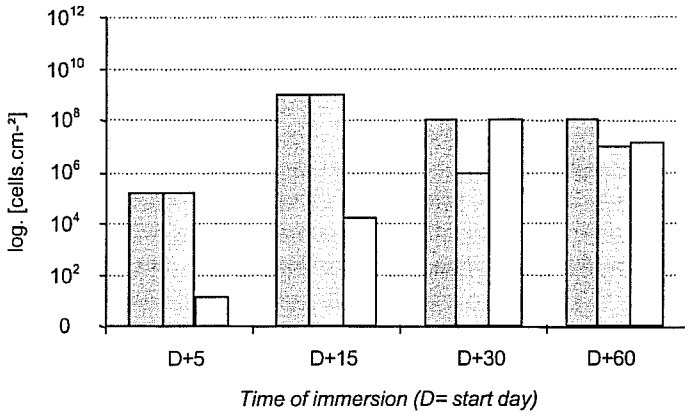

Fig. 3 : bacterial enumeration (average number) in biofilm. Essay with and without CP in presence of natural SRB and TRB, (60 days of immersion).

The sterile essay, without CP, has shown an ordinary general oxidation (Fig 4). On the opposite after 30 days of exposure in seawater containing bacteria, an important microbiologically influenced corrosion was observed in picture 5 and in picture 6 (black "crust", H_2S odour, metal aspect with deep pit under crust, SEM observation of bacteria on pit and FeS analysis).

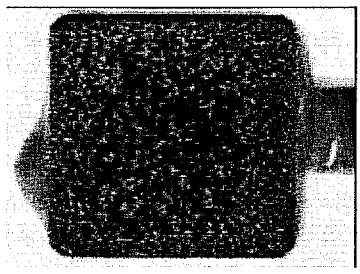

Fig. 4: surface oxidation in sterile seawater, without CP (30 days of exposure)

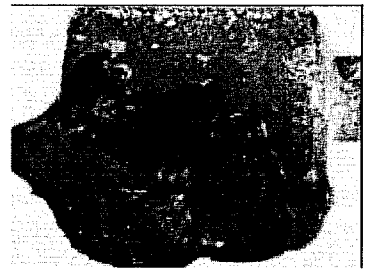

Fig. 5: external aspect with crust in presence of SRB/TRB natural strains, without CP (30 days of exposure)

Fig. 6: surface aspect (pits) under crust formed by SRB/TRB natural strains, without CP (30 days of exposure)

No pits are identified under CP. However, after stopping the CP, a corrosion phenomenon was observed under the non-uniform deposit, which covers the electrodes. Picture 8 shows one sample tested in seawater containing SRB and TRB. In this case, the calcareous deposit observed is compact and stable in comparison with the crumbly calcareous deposit obtained in sterile seawater (Fig.7). This result illustrates the structural stability of the deposit in non-sterile seawater.

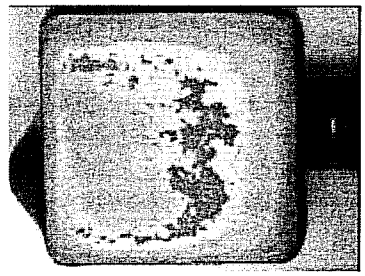

Fig. 7: calcareous deposit aspect (crumbly), CP –1000 mV/ECS in sterile seawater (30 days of exposure)

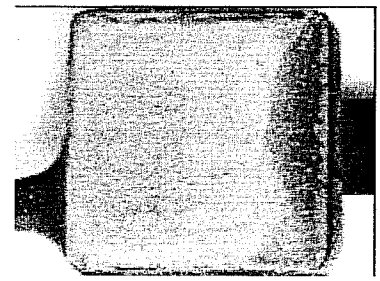

Fig. 8: calcareous deposit aspect (compact), CP –1000 mV/ECS in presence of SRB/TRB (30 days of exposure)

Another experiment was conducted in seawater without suspended substances (seawater filtred with 0.2 µm Millipore® filter). We have applied a CP (-1000 mV/SCE) during 25 days and finally stopped the CP during 25 days (failure system simulation). The first results have shown a calcareous growth smaller than the essays with suspended substances, after 25 days of CP. When the CP was stopped, we have observed in presence of bacteria (natural strains), several "crust" development after 15 days of exposure. Without bacteria we have also observed several corroded areas on the edges of the sample and where the calcareous deposit was not homogenous (top of coupon).
As other authors have previously shown [1][4][9], these results confirm the protecting effect of homogenous calcareous deposit and the importance of suspended substances for this formation. The stopping of the CP seems to induce an important bacterial activity in the biofilm.
It is possible that the bacteria have found a better growth condition on the steel surface than in medium for enzymatic processes implicated in MIC.

Strains of Desulfovibrio halophilus and Dethiosulfovibrio peptidovorans

Pictures 9 and 10 show the results of the counts of *Desulfovibrio halophilus* and *Dethiosulfovibrio peptidovorans* respectively settled on carbon steel under various levels of CP. It can be noticed that the number of *D. peptidovorans* (TRB) attached are more important than *D. halophilus* (SRB).
In the case of SRB the cell numbers are less numerous with –1000 mV/SCE with –900 mV/SCE and without CP. In both cases (SRB and TRB) the cell number, in absence of CP, is similar or higher than in CP essays. This is in agreement with the results obtained with natural strains.

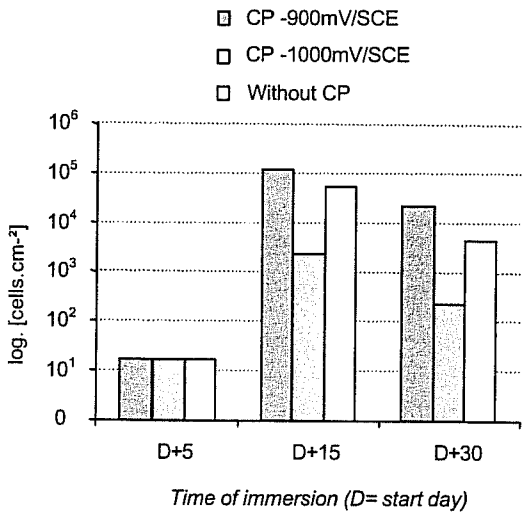

Fig. 9: bacterial enumeration of *Desulfovibrio halophilus* (average number) in biofilm. Essay with and without CP (30 days of immersion).

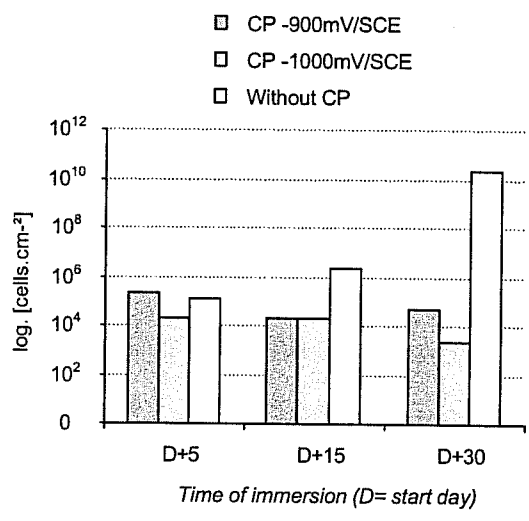

Fig. 10: bacterial enumeration of *Dethiosulfovibrio peptidovorans* (average number) in biofilm. Essay with and without CP (30 days of immersion).

No pits were observed with CP but the MIC observed without CP was not characterised by "crust" but by small pits (Fig. 11). These differences could be due to the bacterial metabolic activity and the medium. SEM images have shown the bacteria attachment near the deposit/biofilm complex (Fig. 12). These complexes seem to be very dense

and to cover the whole surface of the sample. Without CP, we have observed a bacteria accumulation on pits. Picture 13 shows the EDS analysis in and around the pit area. We have observed an important peak of sulphur associated with small oxygen and iron peak. This presence of FeS is a characteristic of sulphide-producing bacteria activities.
Picture 14 shows the comparison of surface EDS analysis between CP and non-CP essays. With CP we can observed that the signal of Fe is cancelled by calcareous deposit signal, whereas without CP, iron and sulphide peaks are observed.

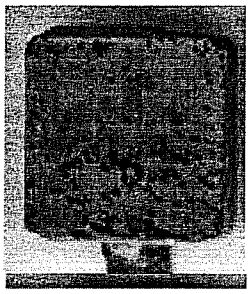

Fig. 11: Macroscopic observation of pits induced by *Dethiosulfovibrio peptidovorans* without CP (30 days of immersion).

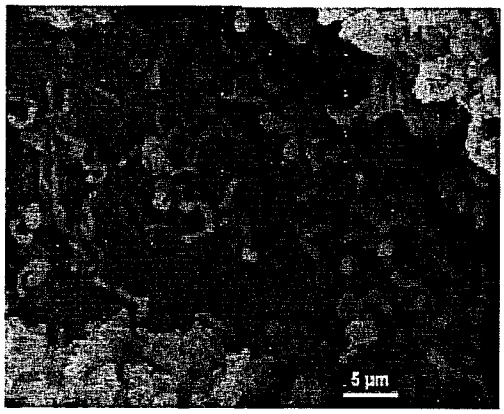

Fig. 12: SEM analysis of steel surface: bacterial colonization near the deposit/biofilm complex. Essay with CP (-900 mV/SCE) in presence of *Dethiosulfovibrio peptidovorans*, (5 days of immersion).

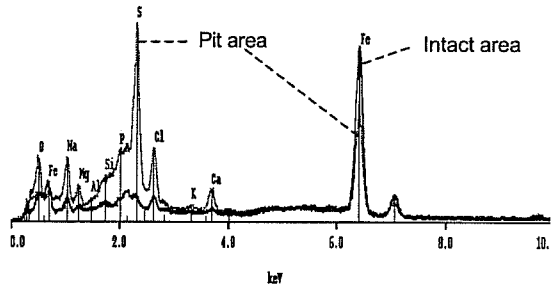

Fig. 13: EDS analysis on and near pit. Essay without CP in presence of *Desulfovibrio halophilus*, (15 days of immersion).

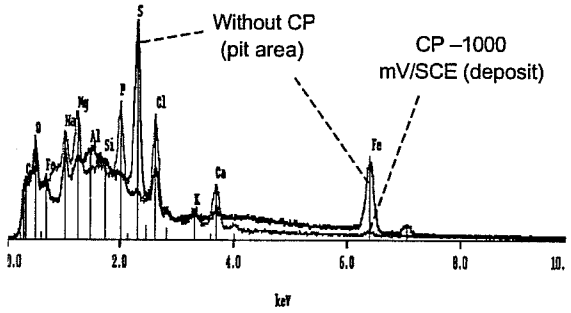

Fig. 14: EDS analysis of steel surface. Essay with and without CP in presence of *Desulfovibrio halophilus*, (15 days of immersion).

With and without CP, we have enumerated a similar cell number in biofilm. These experiments have indicated the influence of CP on the bacterial activity. As the work with pure strains, we are sure to count one sole species, thus the same original metabolic and enzymatic capacities. Therefore, these bacteria seem to inhibit corrosion.

However, other hypotheses are possible: The absence of FeS does not necessarily mean that there has been a modification of the bacterial metabolism. As a matter of fact, bacteria may produce sulphides, which, without Fe^{2+}, will not be able to produce FeS.

CONCLUSIONS AND PROSPECTS

Under the condition described above, the CP does not prevent the biofilm formation of SRB and TRB but increases their growth during the initial stage. After several weeks, without CP, the number of bacteria in the biofilm is similar to the one observed with CP. On the one hand, bacteria increase the protecting role of calcareous deposits by improving their density and structural stability. On the other hand, CP could change the bacterial metabolism (no MIC is observed under CP). Other sample tests should be done in order to confirm the [Fe] hydrogenase implication or other metabolic ways.
Our work does confirm the CP efficiency to prevent carbon steel corrosion in marine environment. Nevertheless, the failure simulation could accelerate the MIC phenomenon, especially where non-homogenous calcareous covered areas exist.

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