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ORIGINAL PAPER

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

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Abstract The industrial crops of microalgae use processes calling upon the presence of parts of metal nature such as steel 316L type. The goal of this study is to test the electrochemical behavior of this material in a marine culture of microalgae. *Porphyridium purpureum* was used under a photoperiod of alternation darkness/light 12/12 h, in order to apprehend the problems of biocorrosion involved in the biofouling. The evolution of the free potential of corrosion, according to the position of the samples and for different surface roughness, observations of the surface quality under the electron microscope with sweeping were carried out. The results showed that, overall, the strain *P. purpureum* does not have a corrosive effect on the 316L. The free potential of corrosion lies between −0.307 and −0.005 V(SCE). The adhesion of the cells seems stronger on the interface air/solid of the half-plunged sample with surface grit polished 1,000, confirmed by the presence of biofilm on the air part. The photoperiod acts on the evolution of the generated free potential of corrosion of the one 24-h period oscillation. Furthermore, the samples plunged horizontally lead to a stabilizing effect on the potential of free corrosion.

Keywords Microalgae · Biocorrosion · Adhesion · Steel 316L · *Porphyridium purpureum*

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Abbreviations

EPS	Extracellular polymeric substances
HALP1000	Half-plunged rugosity 1,000
HALP 500	Half-plunged rugosity 500
HORP1000	Horizontally plunged rugosity 1,000
HORP 500	Horizontally plunged rugosity 500
MA	Medium with algae
MIC	Microbiologically influenced corrosion
MS	Sterile medium
OCP	Open circuit potential
SCE	Saturated calomel electrode
VERTP1000	Vertically plunged rugosity 1,000
VERTP500	Vertically plunged rugosity 500

Introduction

Microalgae, like the majority of micro-organisms, have a strong tendency to colonize surfaces with which they are in contact. The formation of biofilms not only poses problems of contamination due to the resistance of the microalgal cells to physical elimination during cleaning but will also induce local physicochemical conditions, possibly leading to problems of deterioration or corrosion. Products result from biocorrosion or MIC (microbiologically influenced corrosion) being able to generate modifications in the characteristics of the products, if they are used at agroalimentary or pharmaceutical ends. It is necessary to add to that the losses of biomass that can be generated by the formation of these biofilms. In all of these cases, adhesion is undesirable. On another side, often, in the industrial crops, one may find it beneficial to optimize the parameters of culture for a better output, then the immobilization of the microalgaes was studied for various algal species on supports of various natures [1, 21, 24]. Few articles have

reported about phenomena of immobilization of microalgae on steels and some others treated the phenomena of adhesion or colonization of diatoms or chlorella on stainless steels [3, 7, 20, 23].

The strain that was the subject of our study is *Porphyridium purpureum*, also described as *Porphyridium cruentum purpureum* or *Porphyridium marinum.* It is a marine microalga that is very exploited in biotechnology for its metabolic capacities of production of extracellular polymeric polysaccharides (EPS) of pigments and antioxidants. It is among the most studied strains; however, implementation of the industrial culture of these microalgae and then the extraction of its products can be confronted with various problems of order practices such as biocorrosion and deterioration of materials or fixing of the cells on the supports whatever their nature may be.

In this study, a mild steel of 316L type, largely used in the bioreactors in the agroalimentary field in a general way and in certain industrial photobioreactors in particular, was used. The stainless steels are often used for their properties of corrosion resistance. However, in spite of their character of inoxidizability, these alloys are not completely immunized against corrosion and degradations by punctures or caves are frequently observed. Of electrochemical nature, these forms of corrosion are controlled by oxygen: its reduction on the surface of the stainless steels constitutes the "indeed; determinant motor" of their corrosion [17].

In the photobioreactors, the stainless steel is present in the form of metal parts, a stirrer for example [9] or probes of measurement. This alloy can also be used in the composition of the materials like steel sheets, which are used in the stages of extractions of oil in mechanical–biological energy manufacturing systems [2]. The surface quality (like texture) of the metal parts can undergo modifications being able to support or not the adhesion of the cells [2]. Sterilization can also modify the properties of surfaces of the materials, which could influence the adhesion capacity of the cells [22].

The study of the adhesion phenomenon of the microalgal cells is essential because it enables us to define its reversible or irreversible nature as well as the parameters that condition it. In our study, the concerned parameters are the position of the metal support and its roughness. Although these two parameters are necessary, they are not sufficient to explain the force of adhesion of the cells. The parameters which influence the adhesion of the microalgal cells are mainly the hydrophobicity of the substrate and that of the cell, the age and the cellular concentration, as well as the conditions of culture (nutritional availability, mode of flow, physico-chemical conditions: pH, oxygen concentration, presence of bacteria or not) [23].

The role of the microalgae biofilms studied for their action of biocorrosion is in general ignored, and in any case, little studied, and on the impact of condition exposure of working electrode in nature of a biofilm adhesion and in open circuit potential (OCP) evolution.

The aim of this work is to study the constitution of a biofilm being based on algal strain and its possible corrosion effect on stainless steel 316 type material. Three aspects were investigated:

- Evolution of the free potential of corrosion or open circuit potential OCP that is being generally used to estimate the risk of corrosion of metallic materials [6].
- Assessment of the adherent algal cells.
- Follow-up of the growth and the evolution of the pigments produced by *P. purpureum.*
- These last parameters are correlated directly with the evolution of the free potential of corrosion.

Materials and methods

Strain used

The strain of *P. purpureum* is registered as Ro108. It comes from the collection Sammlung von Algenkulturen Pflanzenphysiologister, Institut der Universität Göttingen (Germany) where it is referred SAG 1380.

Culturing strain

Starting from a preculture whose absorbance is 1.2, 400 ml of new culture (dilution 10% v/v) was prepared in a 500-ml reactor. The encountered problems, apart from the biofouling, in the photobioreactors, are the proliferation of the opportunist germs such as the bacteria, mushrooms, protozoa, being able to obstruct the growth of the microalgaes. All the precautions to avoid all the contaminations and selected working conditions in closed system were chosen. It should be noted that all handling relating to the setting in algal culture was carried out under sterile conditions. Operating conditions: Temperature: not controlled, illumination: mode light darkness 12 h/12 h; incidental light intensity: 45 µEinstein. m^2 s^{-1}; culture medium: [14]; % CO_2: 0.03%; pH: 7.

Tested material

A stainless mild steel of austenitic stainless steel, 316L type Ref: Radiospares (Beauvais, France) was used.

Samples preparation

Six steel rectangular samples (30 × 10 mm) were used. To make sure a surface of precise study, each sample was moulded in a resin on only one face, the second being

naked and being used as electrode of measurement. Two types of roughness were chosen according to the last rank of polishing used (500 and 1,000) in order to see the effect of roughness on the evolution of the free potential of corrosion and the adhesion of the cells; the samples were polished gradually using discs of emery paper of various gauges, degreased with alcohol, rinsed with the distilled water, dried for a few seconds with compressed air, and then stored in a desiccator.

Experimental procedure

Device description

The experimental device corresponds to an electrochemical reactor comprising several entries: an entry for the samples, an entry for the silicone autoclavable pipes being used for the bullage (entered and left the filtered air), an entry allowing sampling, distilled water addition and adjustment of the pH, an entry for the electrode of reference; the opening of the bioreactor is carried out using carded cotton wrapped in gauze; this system is agitated in a magnetic way. The reactor containing the samples and the culture medium were sterilized separately at 121°C for 20 min. The electrode of reference was sterilized beforehand using a distilled water/alcohol mixture with 70% ethanol for 15 min. The electrode was then introduced aseptically under a hood and in the presence of the flame, as well as the algal culture in the electrochemical cell.

Position of the samples and nomenclature

Each sample is defined by its position, compared to the bottom of the engine, and its roughness. The position of the samples within the engine is done in three ways for each type of surface roughness. The samples are thus named: two samples vertically placed and completely immersed: VERTP500, VERTP1000, two samples vertically placed, of which the half only is immersed in the culture medium and which were marked beforehand: HALP500, HALP1000. Two samples horizontally placed and completely immersed: HORP500, HORP1000. The medium of immersion was specified: MA, MS, respectively medium with algae and sterile medium.

Experimental measurements

Measure of absorbance

The evolution of the optical density at 760 nm for the growth of microalgae as well as the evolution of the production of the pigments: 683 nm chlorophyll *a*, 565 nm, phycoérythrin, 450 nm for carotenoid [4] were followed using a spectrophotometer BECKMAN DU-64.

Measure of the free potential of corrosion

The follow-up of the variation of the free potential of corrosion was ensured using a multiplexer HP AGILENT34970A, which corresponds to a power station of acquisition governed by Benchlink Datalogger software (Agilent Technologies Hewlett-Packard Company, Santa Clara, California, USA). This multiplexer was connected to the six electrodes of measurement and a saturated calomel electrode of reference. The results were expressed in volts compared to the electrode of reference/SCE.

Measure of abiotic factors and readjustment of various parameters

Daily, and under aseptic conditions, approximately 1 ml of culture was taken off in order to follow the absorbance at different wavelengths. The culture medium was readjusted at the initial level using sterile distilled water. The pH was measured daily as well as the temperature. The pH was given to an adequate value using a diluted hydrochloric acid solution in order to not exceed the extreme value of 8.

The measurement of the incidental and outgoing light intensity was carried out using a photometer LI-COR 190SA. The quantity of light was adjusted, progressively, according to the quantity of absorptive light, which was proportional to the quantity of algal biomass. After 10 days of culture, a third lamp was added, which gave a total incidental light of 45 μ moles.m^{-2}.

Termination of the experiment and withdrawal of the samples

At the end of 40 days of experimentation, the samples were withdrawn and rinsed with sterile salt water.

Assessment of the adherent algal cells to the metal support

The samples were put in a beaker containing 25 ml of salted water (20 g l^{-1}NaCl solution) and passed at the ultrasound bath for 10 min (20 min for the half-immersed samples, the microalgae being more strongly attached). Cell density (number of cell ml^{-1}) was measured by microscope counting in a Malassez hemocytometer. Three series of counting were carried out in the solution for each sample with an optical microscope (Axioplan Zeiss, Germany) and the average was considered.

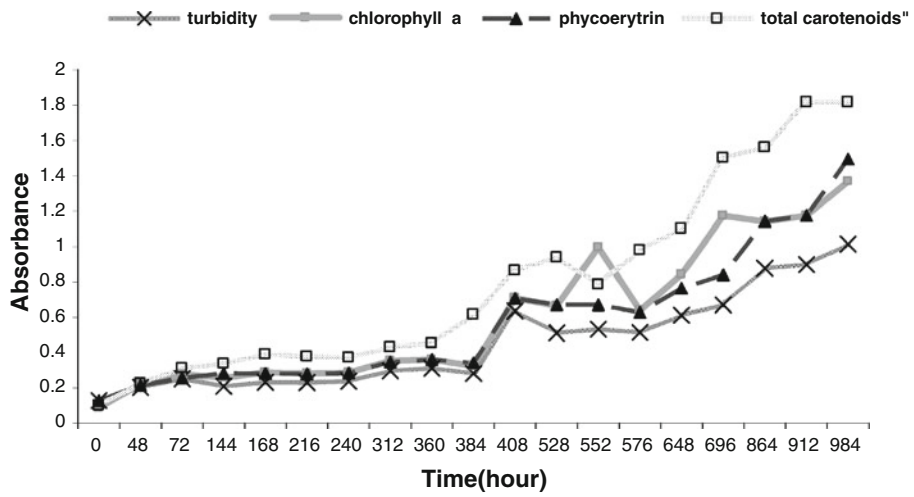

Fig. 1 Evolution of the growth and the production of the pigments

Microscopic study

The samples were observed under electron SEM microscope (Jeol SM T220-A-1) after metallization, under various angles, and various enlargements.

Results and discussion

Growth evolution

Figure 1 (turbidity curve) shows two phases: a long lag phase, which lasts approximately 384 h, and a phase of exponential growth, which, in our opinion, is not finished, and arrives at a maximum value of optic density approximately at 1. This slow growth is probably due to the very low initial absorbance value of 0.1 and to the light conditions that are not optimal. In fact, the fast growth of *Porphyridium* was obtained when the culture was grown under continuous-light conditions [26].

The choice of starting the culture on a low initial absorbance value was made in order to determine the various phases of *Porphyridium* growth i.e., the lag phase, the exponential phase, and the stationary phase. This was done to establish a correlation between the evolution of the potential of corrosion and the metabolic phases of *Porphyridium*. The shape of the production curves of the various pigments (total phycoerythrin, carotenoids, and chlorophyll-*a*) is similar to that of the growth curve. A clear increase is noticed in the concentration of the various pigments during the phase of exponential growth, in particular the carotenoids.

Free potential of corrosion evolution

Figure 2 shows that the free potential of corrosion in the presence of algae varied between −0.307 and −0.005 V, approximately. The comparison of the samples in all positions considered shows two extremes, the sample in position half plunged, polished with 1000(HALP1000), which moves away from the others and shows a potential close to 0 and the sample polished with 500 (HALP 500), and being in the same position which, records the lowest potential to him, approaching −0.3 V.

Roughness is a parameter that influences, in theory, the rate of cellular adhesion and the free potential of corrosion. The results obtained with two different samples for the same position and different roughness were compared to the immersed samples with half, the samples polished at 1,000, records apotential that variesbetween−0.152 and −0.005 V, approximately, which is higher compared to the sample polished with 500, which records an active potential of −0.281 with −0.074 V, as shown in Fig. 3a. The examination of the shape of the two curves shows two successive chronological stages, which are: 0–400 h and 400–820 h. If the stages are common to both coupons, it is not the same for the behavior of the latter during these phases. For the sample polished with 1,000, the potential starts with values close to zero, followed by a reduction. The potential then increases to be stabilized until the end of the experiment to a slightly negative value. The sample polished with 500 exhibited a potential lower than that of the sample polished at 1,000. This potential then decreases gradually to stabilize itself between −0.25 and −0.30 V at the end of the experiment.

Another important parameter is the nature of the steel chosen; the 316L is corrosion-resistant often even under the action of the bacteria. Because of its chemical composition, the low values of recorded potential are not astonishing, but this material is also defined by its surface quality, topography, roughness, and hydrophobicity. Rough surfaces of the passivated stainless steels are exposed to corrosion, which leads to puncture and cracks that smooth surfaces [15].

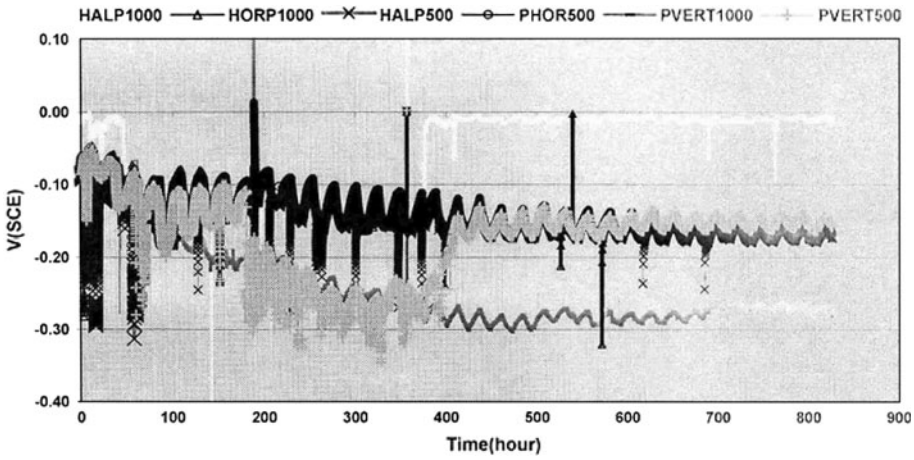

Fig. 2 Evolution of the free potential of corrosion for all samples in the presence of algae

The influence of surface roughness on the adhesion of microbes is still under debate. It seems that the dominant trend is to assume that roughness values of the order of the size of bacterial cells favor bacterial settlement [8]. Sekar et al. [23] showed in their studies that microalgae attachment was higher in a rough surface than in a smooth surface in case of titanium and stainless steel.

In our study, roughness especially influences the phenomenon of adhesion—the number of adherent cells noted for the polished samples with 500 is lower than that recorded for the polished samples with 1,000, regardless of the position of the sample. This is not a trivial result, as the lower the rugosity, the higher the number of immobilized cells.

The measurement of the potential on samples being half-plunged is much more difficult to interpret than that for the immersed samples, the samples here being in contact with two distinct phases—the air and the medium. In addition, the constant variation of the level of liquid (and thus of the ratio of surfaces immersed/emerging surface) is in permanent evolution. The detailed observation of the two curves can however, provide interesting information.

The majority of papers focus on the solid–liquid interface; literature addressing the solid–air interface is considerably less substantial. Attached cells rather than biofilm are therefore found at the solid–air interface as well as the solid–liquid interface. Intermittent exposure of the substratum to moisture, for example during cleaning of hygienic surfaces or external surface exposure to rain, or at a meniscus, generates a solid–liquid–air interface, at which fouling is apparent [25].

We did not encounter in the literature any electrochemical studies referring to the exposure position of the metallic samples, except in one work that tested a copper sample in the position half-plunged in a bacterial culture [16]. Electrochemically, this sample behaves differently than the others. These authors concluded that it is completely possible to obtain different results for the same system metal/solution/microorganism, but differently exposed. In our opinion, the position of the samples especially brings into play the different forces of adhesion for the immersed samples at half, where there exists a gradient of moisture on the surface of the material between the immersed part and the air part. This case is similar to the one described by Haubner et al. [11] when they studied aeroterrestrial microalgae growing in biofilms on facades in the interface between hard substrata and the atmosphere. They concluded that this phenomenon was a response to temperature and water stress. We think that the presence of the adherent cells modifies the free potential of corrosion of a metal in the case of HALPH1000. The curve of potential of this sample is practically confused with those of the completely immersed samples, until approximately 400 h. Beyond this, it separates some and approaches the null potential. The potential curve of the sample of roughness 500 takes a form closer to that recorded for the completely immersed samples. For the samples immersed in driving position, the potential varies with regard to the sample polished at 1,000, between −0.173 and −0.057 V whereas for the sample polished with 500 the potential passes from −0.347 to −0.057 V. For this last, a reduction between time 200 and 400 h, absent in the first case, as shown in Fig. 3b, is noted. Apart from this phase, the two curves are superimposed.

For the samples immersed in the horizontal position, the potential varies approximately between −0.167 and −0.055 V. For the two samples, the two curves are superimposed. There are no notable differences with regard to the two types of roughness, as shown in Fig. 3c. In conclusion, the parameter roughness does not seem to influence the evolution of the potential, except in the case of the immersed samples in the position half-plunged where paradoxically the sample polished with a higher degree (1,000) exhibited a potential definitely more raised that the sample polished with a least degree (500).

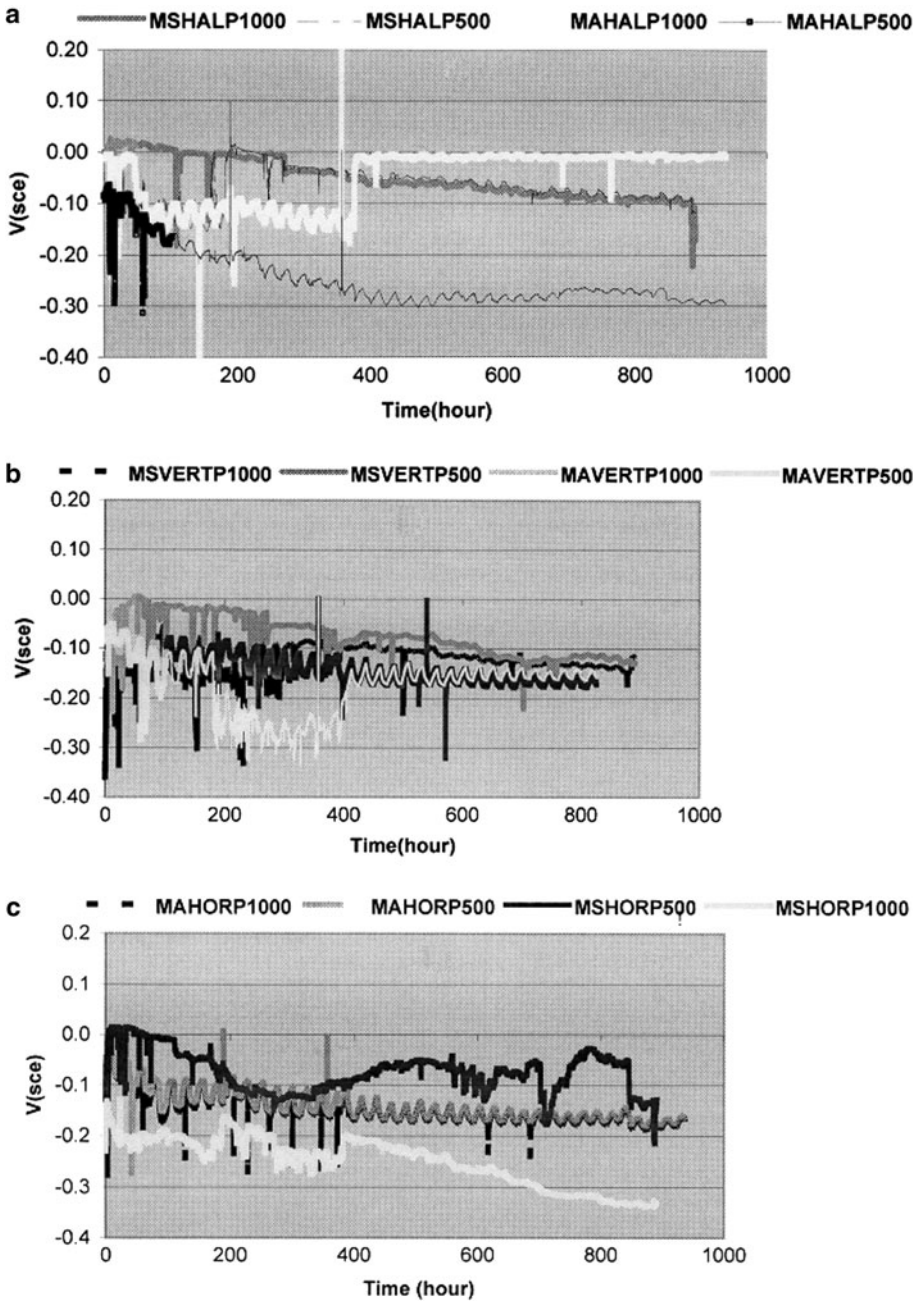

Fig. 3 Evolution of the free potential of corrosion with and without algae. **a** Half-plunged samples. **b** Vertically plunged samples. **c** Horizontally plunged samples

On the other hand, the effect of the photoperiod on the evolution of the free potential of corrosion appears by the presence of pseudo-cyclical swings of approximately 24:00 (Fig. 4), which would confirm the results of Dowling et al. [5]. These authors studied the effect of the photosynthetic biofilms (case of a cyanophyceae *Anabaena* sp.) on the free potential of corrosion of steel. They showed that the variations or oscillations of the free potential of corrosion, connected to the oxidation of the 316L, are possibly due to the photosynthetic biofilms that produce dissolved oxygen under the effect of the light. Maruthumatu et al. [19] showed that the potential is higher during the day than during the night, by measuring the free potential of corrosion of samples of steel immersed out of marine water under conditions of photoperiod 12:00/12 h. They explained that this potential difference noted in the absence and in the presence of light is due to the presence of algae in the biofilm which produce oxygen by the phenomenon of photosynthesis, which can influence the cathodic reaction by the strong partial pressures created near the electrode.

The same phenomenon was observed by Marconnet et al. [18] while following the evolution of the free potential of corrosion with time on samples of steel immersed in fresh water during 18 days in spring. They obtained curves presenting oscillations similar to those obtained in Fig. 7.

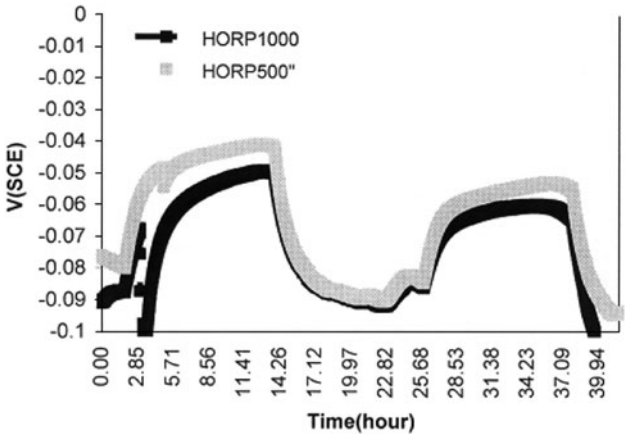

Fig. 4 Oscillation period of OCP in exponential phase of horizontally plunged samples

They charged these oscillations, i.e., the increase and the reduction in the free corrosion potential with the fluctuations of temperature, low night and higher potential of corrosion the day, this one directly related to the light by the phenomenon of photoperiod and the production of oxygen. By comparing their results with those obtained here, it can be deduced that the light that is resulting in a production of photosynthetic oxygen is the determining factor of these oscillations in fresh or marine water.

These swings are variations of potential in the form of more or less homogeneous peaks according to the phase of growth and the position of the sample tested. These peaks appear more homogeneous for the samples in horizontal position and less homogeneous for the sample plunged vertically (Fig. 2).

The stabilization of the free potential of corrosion takes place during the exponential phase, i.e., from 400 h of culture, which coincides with the production of pigments, which have an antioxidative action and would probably cause a passivation of stainless steel and then an anticorrosive action. Heakal et al. [12] and Hefny et al. [13] studied the action of *Dunaliella salina* on the corrosion of titanium and 304L in a saline solution and in sulphate solution they concluded that this stock has an anti-corrosive capacity due to the synthesis of the β-carotene.

The oscillations are more homogeneous during this phase (Fig. 5) than those in the lag phase (Fig. 6). Moreover, it would seem that there is a difference between the pace of the oscillations described above, recorded during the lag phase ranging between 0 and 384 h (Fig. 7), and those noted during the exponential phase (Fig. 6). In this phase, the oscillations of the potential are characterized by regularity and a relative stabilization for all the samples. So we deduced that strain acts by its growth phase in amplitude of these oscillations.

The results obtained in the medium without algae showed that the Hemerick medium is not very corrosive: the potential has a little variation. The cyclical swings miss in medium without algae in all of the samples, even if more or less anarchistic variations are noted. Concerning the half-immersed samples, the potential decreased and stabilized with a constant value of approximately −0.10 V (Fig. 3a).

In the case of the vertically immersed samples (Fig. 3b), the potential varies between −0.350 and −0.138 V/SCE for PVERT1000 and for sample PVERT500, it is stabilized to −0.110 V/SCE, approximately.

Concerning the horizontally immersed samples (Fig. 3c), the potential of the sample polished with 1,000 is stabilized to −0.06 V, approximately, whereas for that polished to 500, it is stabilized in the lower part of −0.3 V.

Number of adherent cells evolution

The number of adherent algal cells obtained after setback for each sample is shown in Fig. 7. The polished samples

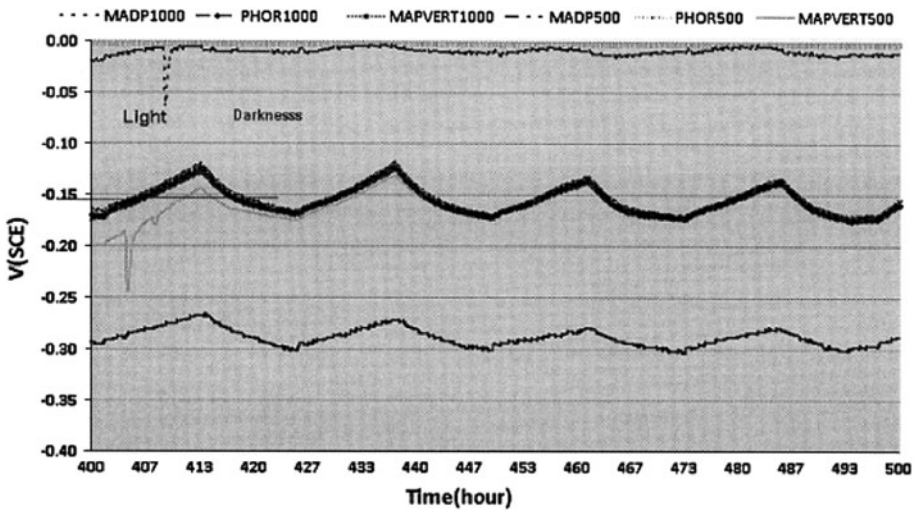

Fig. 5 Oscillation type in the exponential phase

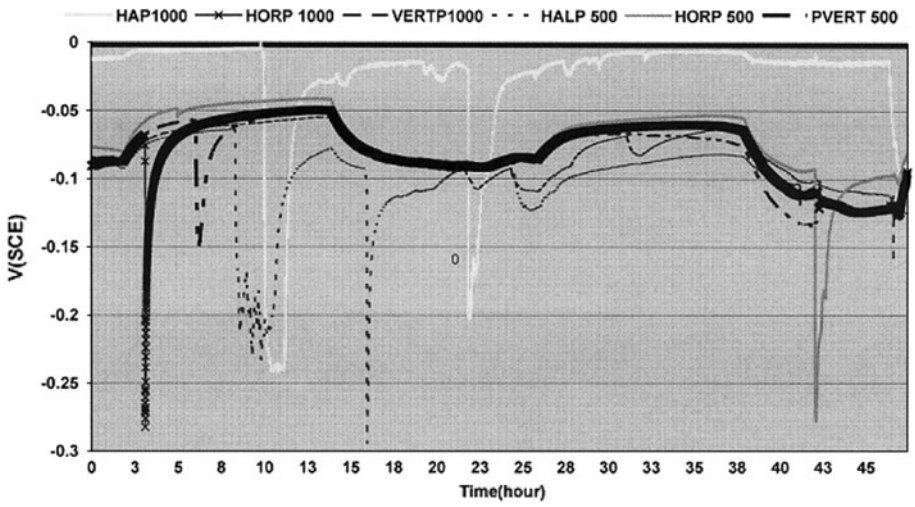

Fig. 6 Oscillation type in the lag phase

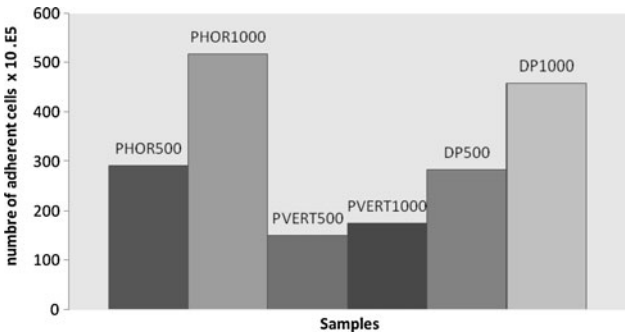

Fig. 7 Number of adherent cells for all samples

with 500 recorded values lower than those obtained for the samples polished at 1,000, the difference being less clear for the samples placed in driving position. It should be recalled that the time of setback by ultrasounds is of 10 min for all the immersed samples. We note that for this time, the number of cells taken down on the immersed vertical samples is definitely lower, for the two types of roughness than that observed for the immersed horizontal samples. For the half-immersed samples, the number of adherent cells is very close to that noted for the samples placed in the horizontal position, and definitely higher than that observed for the samples in the driving position. However, these cells seem more resistant to the setback, because the time that was necessary to use for the sonication is of 20 min instead of 10 min in the other cases. As the cells immobilized on vertical samples is very low, the results obtained on half-immersed samples should be due essentially to the non-immersed part of the samples, and so seem to show that the density of cells immobilized on the non-immersed part of the sample is higher than that obtained even on horizontal samples. A qualitative analysis of the result for HALP500 gives the following estimation: if we deduce the half of cells number obtained for VERTP500 (75 cells) to 283, we obtain 208 cells for the non-immersed half of the surface of the sample, which corresponds to 416 cells if related to the complete surface. The same operation gives the same results for the HALP1000 sample.

Microscopic analysis

For the immersed samples in the position half-plunged, two fields of study were chosen according to the parts observed, the air part and the immersed part. The microscopic examination with sweeping of sample HALP1000 showed that the air part (Fig. 8a) has structures that could be comparable with biological or organic formations or biofilms associated or not with salt crystals; the immersed part presents some salt crystals. The objects observed have the form and the size of the *P. purpureum* cells. That leads to undoubtedly say that all the algae immobilized in this air part were not taken down, even by doubling the time of unhooking. The immersed part presents to surface only stripes (Fig. 8b).

In our case, only the half-plunged sample and polished with 1,000 presents at its surface the forms which would be compared to a biofilm (Fig. 8a) and it is precisely that one which records the relatively highest potential (Fig. 4a). Its behavior seems different compared to the behavior of the samples in other positions. In addition, the qualitative comparison between the numbers of immobilized cells in the three positions showed above that it seemed that the number of cells immobilized on the non-immersed surface of the DP samples was probably significantly higher than those obtained in the other positions. The microalgae present on the surface of gas bubbles are expelled into the air when the bubbles explode at the surface of the liquid. Some land on the non-immersed parts of the samples. They cannot live a long time without water. So, they probably secrete EPS to stick to the surface of the sample and, by building progressively a strongly fixed biofilm, catch the droplets of water splashed

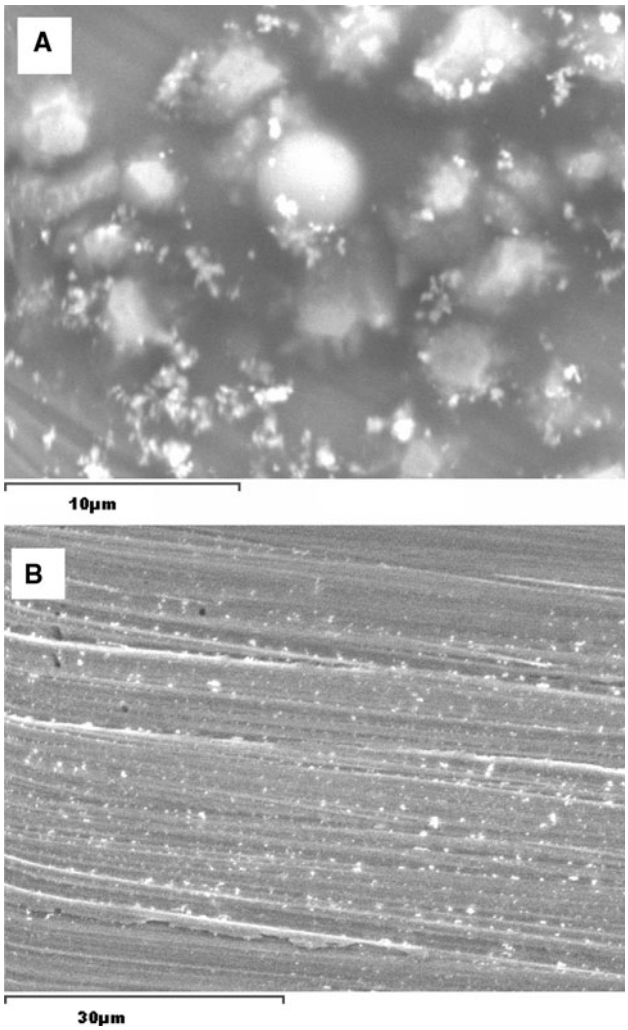

Fig. 8 Results of SEM observations. **a** Porphyridium biofilm resistant to inhooking after 20 min, air part. **b** Immersed part absence of biofilm

by the explosion of the bubbles. These biopolymers are hygroscopic and their presence creates a more hydrated microenvironment in the immediate vicinity of the algal cells, thereby limiting desiccation stress [10].

Conclusions

From the experimental results, the following conclusions can be drawn:

- Overall, a corrosive effect of the stock *P. purpureum* on the 316L was not observed, the free potential of corrosion lies between −0.307 and −0.005 V.
- The adhesion of the cells seems stronger on the interface air/metal in the case of the sample of roughness 1,000 in the position half-plunged, and the density of immobilized cells seems to be higher on the non-immersed part of the samples. These samples behave differently from others.
- The photoperiod probably acts on the evolution of the free potential of corrosion by the presence of oscillations of approximately 24 h due to the production of oxygen by the microalgae in the luminous phase.
- The samples plunged horizontally have a stabilizing effect on the free potential of corrosion. Finally, stainless steel 316L can be considered as a promising material for design in closed systems for axenic *Porphyridium* pure culture. It has a good hygienic status in short time but what is its behavior for a long time in the same conditions? What would happen in open culture systems in the presence of consortium (bacteria + microalgae)? Would continuous light conditions act like photoperiods? It appears necessary to conduct further experiments to answer these questions. The study should also be supplemented by the consideration and evaluation of the strength of the alga on surfaces, not only of stainless steel but also of other materials like titanium, glass, aluminium, or plastic.

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